(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 883 687 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent: 27.10.2004 Bulletin 2004/44

(51) Int Cl.7: C12N 15/12, C07K 14/705, C07K 16/28, C12N 5/20

(21) Application number: 97904948.3

(86) International application number: PCT/BE1997/000023

(22) Date of filing: 28.02.1997

(87) International publication number: WO 1997/032019 (04.09.1997 Gazette 1997/38)

(54) C-C CKR-5, CC-CHEMIKINES RECEPTOR, DERIVATIVES THEREOF AND THEIR USES

CC-CHEMOKINZEPTOR C-C CKR-5, DESSEN DERIVATE UND VERWENDUNGEN

RECEPTEUR-5 DE CHIMIOKINE CC, RECEPTEUR DE CHIMIOKINES CC, LEURS DERIVES ET LEURS APPLICATIONS

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: 01.03.1996 EP 96870021
06.08.1996 EP 96870102

(43) Date of publication of application: 16.12.1998 Bulletin 1998/51

(83) Declaration under Rule 28(4) EPC (expert solution)

(60) Divisional application:
01127469.3 / 1 199 360
04018812.0

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Printed by Jouve, 75001 PARIS (FR)

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U.S. Serial No. 09/464,902
Filed: December 16, 1999
Exhibit 31

Description

Field of the present invention.

[0001] The invention provides methods for determining anti-ligands capable of inhibiting ligand binding to peptides of a new chemokine receptor. The invention also relates to screening for drugs binding specifically to said peptides and treatments involving said peptides or the nucleic acid molecules encoding said peptides.

Technological background and state of the art.

[0002] Chemotactic cytokines, or chemokines, are small signalling proteins that can be divided in two subfamilies (CC- and CXC-chemokines) depending on the relative position of the first two conserved cysteines. Interleukin 8 (IL-8) is the most studied of these proteins, but a large number of chemokines (Regulated on Activation Normal T-cell Expressed and Secreted (RANTES), Monocyte Chemoattractant Protein 1 (MCP-1), Monocyte Chemoattractant Protein 2 (MCP-2), Monocyte Chemoattractant Protein 3 (MCP-3), Growth-Related gene product α (GROα), Growth-Related gene product β (GRO)β, Growth-Related gene product γ (GROγ), Macrophage Inflammatory Protein 1 α (MIP-1α) and β, etc.) has now been described [4]. Chemokines play fundamental roles in the physiology of acute and chronic inflammatory processes as well as in the pathological dysregulacions of these processes, by attracting and simulating specific subsets of leucocytes [32]. RANTES for example is a chemoattractant for monocytes, memory T-cells and eosinophils, and induces the release of histamine by basophils. MCP-1, released by smooth muscle cells in arteriosclerotic lesions, is considered as the factor (or one of the factors) responsible for macrophage attraction and, therefore, for the progressive aggravation of the lesions [4].

[0003] MIP-1α, MIP-1β and RANTES chemokines have recently been described as major HIV-suppressive factors produced by $CD8^+$ T-cells [9]. CC-chemokines are also involved in the regulation of human myeloid progenetor cell proliferation [6, 7].

[0004] Recent studies have demonstrated that the actions of CC- and CXC-chemokines are mediated by subfamilies of G protein-coupled receptors. To date, despite the numerous functions attributed to chemokines and the increasing number of biologically active ligands, only six functional receptors have been identified in human. Two receptors for interleukin-8 (IL-8) have been described [20, 29]. One (IL-8RA) binds IL-8 specifically, while the other (IL-8RB) binds IL-8 and other CXC-chemokines, like GRO. Among receptors binding CC-chemokines, a receptor, designated CC-chemokine receptor 1 (CCR-1), binds both RANTES and MIP-1α [31], and the CC-chemokine receptor 2 (CCR2) binds MCP-1 and MCP-3 [8, 44, 15]. Two additional CC-chemokine receptors were cloned recently : the CC-chemokine receptor 3 (CCR3) was found to be activated by RANTES, MIP-1α and MIP-1β [10]; the CC-chemokine receptor 4 (CCR4) responds to MIP-1, RANTES and MCP-1 [37]. In addition to these six functional receptors, a number of orphan receptors have been cloned from human and other species; that are structurally related to either CC- or CXC-chemokine receptors. These include the human BLR1 [13], EBI1 [5], LCR1 [21], the mouse MIP-1 RL1 and MIP-1 RL2 [17] and the bovine PPR1 [25]. Their respective ligand(s) and function(s) are unknown at present.

Summary of the invention

[0005] In one embodiment, the present invention is directed to a method for determining whether an anti-ligand is capable of inhibiting the binding of a ligand, said ligand being an HIV virus or a portion thereof, to a peptide having an amino acid sequence which presents more than 80% homology with SEQ ID NO. 2 shown in figure 1, said method comprising the steps of contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said peptide, with the anti-ligand under conditions permitting a binding of the anti-ligand to said peptide and determining whether the said anti-ligand inhibits the binding of the said ligand, to said peptide.

[0006] In a second embodiment, the present invention relates to a method for determining whether an anti-ligand is capable of inhibiting the binding of a ligand, said ligand being an HIV virus or a portion thereof, to a peptide having an amino acid sequence which presents more than 80% homology with SEQ ID NO. 2 shown in figure 1, said method comprising the steps of:

- preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said peptide,
- isolating a membrane fraction from the cell extract,
- contacting the anti-ligand with the membrane fraction under conditions permitting binding of the anti-ligand to said peptide, and
- determining whether the said anti-ligand inhibits the binding of the said ligand to said peptide.

1β chemokine at a concentration less or equal to 10 nm, and is advantageously also stimulated by the MIP-1α or RANTES chemokines. However, said chemokine receptor is not stimulated by the MCP-1, MCP-2, MCP-3, IL-8 and GROα chemokines.

[0020] In addition, the peptide to be used in the methods according to the invention or a portion thereof is also a receptor of HIV viruses or a portion of said HIV viruses.

[0021] It is meant by "HIV viruses", HIV-1 or HIV-2 and all the various strains of HIV viruses which are involved in the development of AIDS. It is meant by a "a portion of HIV viruses", any epitope of said viruses which is able to interact specifically with said receptor. Among said portions of viruses which may be involved in the interaction with the peptide as defined in the invention, are peptides encoded by the ENV and GAG viruses genes.

[0022] According to the methods of the present invention as defined above, said HIV virus may be selected from the group consisting of the human immunodeficiency virus 1 (HIV 1), the human immunodeficiency virus 2 (HIV 2) or a portion of said HIV viruses.

[0023] Preferably, said portion of HIV viruses is the glycopeptide gp120/160 (membrane-bound gp160 or the free gp derived therefrom) or a portion thereof.

[0024] It is meant by a "portion of the glycopeptide gp120/160" any epitope, preferably an immuno-dominant epitope, of said glycopeptide which may interact specifically with the peptide as defined in the invention, such as for instance the V3 loop (third hypervariable domain).

[0025] The methods of the present invention as defined above may further comprise the step of measuring the infectivity of the cell by an HIV strain wherein said anti-ligand, drug, agonist or antagonist decreases infectivity by said HIV strain. In said method the cell may be a lymphocyte cell. In addition according to the present invention, the HIV strain may be the human immunodeficiency virus 1 (HIV-1) or the human immunodeficiency virus 2 (HIV-2). The invention further specifies that the decrease in HIV infectivity may be measured by the dosage of an HIV protein. Preferably, said HIV protein is the HIV antigen P24.

[0026] Advantageously, the peptide to be used in the methods according to the invention is a human receptor.

[0027] The present invention concerns also methods involving the use of a nucleic acid molecule having more than 80%, preferably more than 90%, homology with the nucleic acid sequence of SEQ ID NO. 2 shown in the figure 1.

[0028] Preferably, said nucleic acid molecule has at least the nucleic acid sequence shown in SEQ ID NO. 2 of figure 1 or a portion thereof.

[0029] It is meant by a "portion of said nucleic acid molecule" any nucleic acid sequence of more than 15 nucleotides which could be used in order to detect and/or reconstitute said nucleic acid molecule or its complementary strand. Such portion could be a probe or a primer which could be used in genetic amplification using the PCR, LCR, NASBA or CPR techniques for instance.

[0030] The present invention concerns more specifically methods involving the use of the nucleic acid molecules encoding the peptide to be used according to the invention. Said nucleic acid molecules are RNA or DNA molecules such as a cDNA molecule or a genomic DNA molecule.

[0031] The present invention is also related to methods utilizing a vector comprising the nucleic acid molecule as defined above. Preferably, said vector is adapted for expression in a cell and comprises the regulatory elements necessary for expressing the amino acid molecule in said cell operatively linked to the nucleic acid sequence according to the invention as to permit expression thereof.

[0032] Preferably, said cell is chosen among the group consisting of bacterial cells, yeast cells, insect cells or mammalian cells. The vector according to the invention is a plasmid, preferably a pcDNA3 plasmid, or a virus, preferably a baculovirus, an adenovirus or a semliki forest virus.

[0033] The present invention concerns also the methods involving the use of a cell, preferably a mammalian cell, such as a CHO-K1 or a HEK293 cell, transformed by the vector as defined above. Advantageously, said cell is non neuronal in origin and is chosen among the group consisting of CHO-K1, HEK293, BHK21, COS-7 cells.

[0034] The present invention also concerns the use of a cell (preferably a mammalian cell such as a CHO-K1 cell) transformed by the vector as defined above and by another vector encoding a protein enhancing the functional response in said cell. Advantageously, said protein is the Gα15 or Gα16 (G protein, α subunit). Advantageously, said cell is the cell CHO-K1-pEFIN hCCR5-1/16.

[0035] The invention concerns also the use of an antisense oligonucleotide having a sequence capable of specifically hybridising to an mRNA molecule encoding the peptide as defined above so as to prevent translation of said mRNA molecule or an antisense oligonucleotide having a sequence capable of specifically hybridising to the cDNA molecule encoding the peptide according to the invention.

[0036] Said antisense oligonucleotide may comprise chemical analogs of nucleotide or substances which inactivate mRNA, or be included in an RNA molecule endowed with ribozyme activity.

[0037] The present invention thus relates to the use of an antisense oligonucleotide, comprising a sequence which specifically hybridizes to a nucleic acid molecule having more than 80% homology with nucleic acid sequence SEQ ID NO. 2 shown in figure 1, for the manufacture of a medicament in the prevention and/or the treatment of an infection

by the human immunodeficiency virus 1 (HIV-1) and/or the human immunodeficiency virus 2 (HIV-2) (AIDS).

[0038] The present invention also relates to the use of an antisense oligonucleotide, comprising a sequence which specifically hybridizes to a nucleic acid molecule having at least the nucleic acid sequence SEQ ID NO. 2 shown in figure 1 or a portion thereof, for the manufacture of a medicament in the prevention and/or the treatment of an infection by the human immunodeficiency virus 1 (HIV-1) and/or the human immunodeficiency virus 2 (HIV-2) (AIDS).

[0039] Preferably, said nucleic acid molecule encodes a peptide as defined above. Furthermore, said nucleic acid molecule may be a cDNA molecule or a genomic DNA molecule. In addition, according to the present invention said antisense oligonucleotide may comprise chemical analogs of nucleotides.

[0040] Another aspect of the present invention concerns a method as defined above using a ligand or an anti-ligand (preferably an antibody) other than known "natural ligands", which are chosen among the group consisting of the MIP-1β, MIP-1α or RANTES chemokines, HIV viruses or a portion of said HIV viruses, wherein said ligand is capable of binding to the receptor according to the invention and wherein said anti-ligand is capable of (preferably competitively) inhibiting the binding of said known "natural ligand" or the ligand according to the invention to the peptide according to the invention.

[0041] The present invention further specifies a method according to the invention, wherein the anti-ligand, the drug, the agonist or the antagonist may be an antibody.

[0042] The exclusion in the above identified definition of known chemokines, HIV viruses or a portion of said HIV viruses, does not include variants of said "natural" viruses or said "natural" portion which may be obtained for instance by genetic engineering and which may mimic the interaction of said viruses and portion of said viruses to the peptide according to the invention.

[0043] Advantageously, said antibody to be used in a method of the invention is a monoclonal antibody which is preferably directed to an epitope of the peptide according to the invention and present on the surface of a cell expressing said peptide.

[0044] Preferably, said antibody is produced by the hybridome cell AchCCR5-SAB1A7.

[0045] In a further embodiment, the present invention relates to an antibody which inhibits or reduces the binding of the human immunodeficiency virus 1 (HIV 1) or the human immunodeficiency 2 (HIV 2) to a peptide having an amino acid sequence presenting more than 80%, 90% or 95% homology with SEQ ID NO. 2 shown in figure 1, identified according to a method of the present invention. Alternatively, the amino acid of the peptide may have an amino acid sequence as presented in SEQ ID NO. 2 of figure 1. Said antibody according to the invention may be a monoclonal antibody, preferably a monoclonal antibody directed to an epitope of the said peptide, present on the surface of a cell expressing said peptide.

[0046] According to the invention, the antibody as described above may decrease the infectivity of a cell by an HIV strain. According to a preferred embodiment, said cell is a lymphocyte cell. The HIV strain recognized by the antibody of the present invention may be a human immunodeficiency virus 1 (HIV-1 strain) or a human immunodeficiency virus 2 (HIV-2 strain).

[0047] The invention concerns also the pharmaceutical composition comprising an effective amount of the above identified antibody. The pharmaceutical composition comprises also a pharmaceutically acceptable carrier, preferably capable of passing through said cell membrane.

[0048] Preferably, the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by cell after binding to the structure. The structure of the pharmaceutically acceptable carrier in said pharmaceutical composition is capable of binding to a receptor which is specific for a selected cell type.

[0049] Said pharmaceutical composition according to the invention may be used for the manufacture of a medicament in the prevention and/or the treatment of an infection by the human immunodeficiency virus 1 (HIV-1) and/or the human immunodeficiency virus 2 (HIV-2) (AIDS).

[0050] The invention relates to a method as defined above wherein detecting by means of a bio-assay, is by means of a modification in a second messenger concentration (preferably calcium ions or inositol phosphates such as IP_3) or a modification in the cellular metabolism (preferably determined by the acidification rate of the culture medium).

[0051] Preferably, the second messenger assay to be used according to the invention comprises measurement of calcium ions or inositol phosphates such as IP_3.

[0052] Preferably, the cell used in the methods according to the invention is a mammalian cell non neuronal in origin, such as CHO-K1, HEK293, BHK21, COS-7 cells.

[0053] The present invention concerns also the pharmaceutical composition which comprises an effective amount of an antibody against the peptide according to the invention, effective to reduce the activity of said peptide and a pharmaceutically acceptable carrier.

[0054] It is meant by "an agonist or an antagonist of the peptide to be used according to the invention", all the agonists or antagonists of the known "natural ligand" of the peptide as above described.

[0055] Therefore, the previously described methods may be used for the screening of drugs to identify drugs which specifically bind to the peptide to be used according to the invention.

[0056] The present invention concerns also the use of the pharmaceutical composition according to the invention for the treatment and/or prevention of viral infections of Human Immunodeficiency Viruses 1 and 2 (HIV-1 and 2).

[0057] The deposits of micro-organisms AchCCR5-SAB1A7 and CHO-K1-pEFIN hCCR5-1/16 were made according to the Budapest Treaty in the Belgium Coordinated Collection of Micro-organisms (BCCM), Laboratorium voor Moleculaire Biologie (LMBP), Universiteit Gent, K. L. Ledeganckstraat 35, 3-9000 GENT, BELGIUM.

Short description of the drawings.

[0058]

The figure 1	represents the primary structure of the peptides according to the invention.
The figure 2	represents the amino acids sequence of the active human CCR5 chemokine receptor according to the invention aligned with that of the human CCR1, CCR2b, CCR3 and CCR4 receptors. Amino acids identical with the active CCR5 sequence are boxed.
The figure 3	shows the chromosomal organisation of the human CCR2 and CCR5 chemokine receptor genes.
The figure 4	shows the functional expression of the human active CCR5 receptor in a CHO-K1 cell line.
The figure 5	represents the distribution of mRNA encoding the CCR5 receptor in a panel of human cell lines of haematopoietic origin.
The figure 6	represents the structure of the mutant form of human CCR5 receptor.
The figure 7	represents the quantification of ENV proteins-mediated fusion by luciferase assays.
The figure 8	represents genotyping of individuals by PCR and segregation of the CCR5 alleles in CEPH families.
The figure 9	represents the FACS analysis of sera anti-CCR5 on a CCR5-CHO cell line according to the invention.
The figure 10	represents the inhibition of HIV infectivity with anti-CCR5 antibodies.

Detailed description of the invention.

1. EXPERIMENTALS

Materials

[0059] Recombinant human chemokines, including MCP-1, MIP-1α, MIP-1β, RANTES, IL-8 and GROα were obtained from R & D Systems (London, UK). [^{125}I]MIP-1α (specific activity, 2200 Ci/mmol) was obtained from Dupont NEN (Brussels, Belgium). Chemokines obtained from R & D Systems were reported by the supplier as >97 % pure on SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and biologically active on a bioassay specific for each ligand. The lyophilised chemokines were dissolved as a 100 µg/ml solution in a sterile phosphate-buffered saline (PBS) and this stock solution was stored at -20° C in aliquots. Chemokines were diluted to the working concentration immediately before use. All cell lines used in the present study were obtained from the ATCC (Rockville, MD, USA).

Cloning and sequencing

[0060] The mouse MOP020 clone was obtained by low stringency polymerase chain reaction, as described previously [24, 34], using genomic DNA as template. A human genomic DNA library (Stratagene, La Jolla, CA) constructed in the lambda DASH vector was screened at low stringency [39] with the MOP020 (511 bp) probe. The positive clones were purified to homogeneity and analysed by Southern blotting. The restriction map of the locus was determined and a relevant XbaI fragment of 4,400 bp was subcloned in pBluescript SK+ (Stratagene). Sequencing was performed on both strands after subcloning in M13mp derivatives, using fluorescent primers and an automated DNA sequencer (Applied Biosystem 370A). Sequence handling and data analysis was carried out using the DNASIS/PROSIS software (Hitachi), and the GCG software package (Genetics Computer Group, Wisconsin).

Expression in cell lines

[0061] The entire coding region was amplified by PCR as a 1056 bp fragment, using primers including respectively the *BamHI* and *XbaI* recognition sequences, and cloned after restriction in the corresponding sites of the eukaryotic expression vector pcDNA3 (Invitrogen, San Diego, CA). The resulting construct was verified by sequencing, and transfected in CHO-K1 cells as described [35]. Two days after transfection, selection for stably transfected cell lines was initiated by the addition of 400 µg/ml G418 (Gibco), and resistant clones were isolated at day 10. CHO-K1 cells were cultured using Ham's F12 medium, as previously described [35, 11]. The expression of the active CCR5 receptor in

the various cell clones was evaluated by measuring the specific transcript level by Northern blotting, on total RNA prepared from the cells (see below).

Binding Assays

[0062] Stably transfected CHO-KI cells expressing the active CCR5 receptor were grown to confluence and detached from culture dishes by incubation in phosphate-buffered saline (PBS) supplemented with 1 mM EDTA. Cells were collected by low speed centrifugation and counted in a Neubaeur cell. Binding assays were performed in polyethylene minisorp tubes (Nunc) in a final volume of 200 µl PBS containing 0.2 % bovine serum albumin (BSA) and 10^6 cells, in presence of [^{125}I]-MIP-1α. Non specific binding was determined by addition of 10 nM unlabelled MIP-1α. The concentration of labelled ligand was 0.4 nM (around 100 000 cpm per tube). The incubation was carried out for 2 hours at 4 °C, and was stopped by the rapid addition of 4 ml ice-cold buffer, and immediate collection of cells by vacuum filtration through GF/B glass fiber filters (Whatmann) pre-soaked in 0.5 % polyethyleneinimine (Sigma). Filters were washed three times with 4 ml ice-cold buffer and counted in a gamma councer.

Biological activity

[0063] The CHO-K1 cell lines stably transfected with the pcDNA3/CCR5 construct or wild type CHO-K1 cells (used as controls) were plated onto the membrane of Transwell cell capsules (Molecular Devices), at a density of 2.5 10^5 cells/well in Ham's F12 medium. The next day, the capsules were transferred in a microphysiometer (Cytosensor, Molecular Devices), and the cells were allowed to equilibrate for approximately two hours by perfusion of 1 mM phosphate-buffered (pH 7.4) RPMI-1640 medium containing 0.2 % BSA. Cells were then exposed to various chemokines diluted in the same medium, for a 2 min duration. Acidification rates were measured at one minute intervals.

Northern blotting

[0064] Total RNA was isolated from transfected CHO-K1 cell lines, from a panel of human cell lines of haematopoietic origin and from a panel of dog tissues, using the RNeasy kit (Qiagen). RNA samples (10 µg per lane) were denatured in presence of glyoxal [26], fractionated on a 1 % agarose gel in a 10 mM phosphate buffer (pH 7.0), and transferred to nylon membranes (Pall Biodyne A, Glen Cove, NY) as described [42]. After baking, the blocs were prehybridised for 4h at 42° C in a solution consisting of 50 % formamide, 5x Denhardt solution (1x Denhardt: 0.02 % Ficoll, 0.02 % polyvinylpyrolidone, 0.02 % BSA), 5x SSPE (1x SSPE: 0.18 M NaCl, 10 mM Na phosphate, 1 mM EDTA pH 8.3), 0.3 % Sodium Dodecyl Sulphate (SDS), 250 µg per ml denatured DNA from herring testes. DNA probes were (α^{32}P)-labelled by random priming [14]. Hybridisations were carried out for 12h at 42° C in the same solution containing 10 % (wt/vol) dextran sulphate and the heat denatured probe. Filters were washed up to 0.1x SSC (1x SSC: 150 mM NaCl, 15 mM Na Citrate pH 7.0), 0.1 % SDS at 60° C and autoradiographed at - 70° C using Amersham β-max films.

2. RESULTS AND DISCUSSION

Cloning and structural analysis

[0065] The sequence homology characterising genes encoding G protein-coupled receptors has allowed the cloning by low stringency polymerase chain reaction (PCR) of new members of this gene family [24, 34]. One of the clones amplified from mouse genomic DNA, named MOP020 presented strong similarities with characterised chemokine receptors, sharing 80 % identity with the MCP-1 receptor (CCR2) [8], 65 % identity with the MIP-1α/RANTES receptor (CCR1) [31], and 51 % identity with IL-8 receptors [20, 30]. The clone was used as a probe to screen a human genomic library. A total of 16 lambda phage clones were isolated. It was inferred from the restriction pattern of each clone and from partial sequence data that all clones were belonging to a single contig in which two different coding sequences were included. One of the coding sequences was identical to the reported cDNA encoding the CCR2 receptor [8, 44]. A 4.400 pb *Xba*I fragment of a representative clone containing the second region of hybridisation was subcloned in pBluescript SK+. Sequencing revealed a novel gene, tentatively named CCR5, sharing 84 % identity with the MOP020 probe, suggesting that MOP020 is the mouse ortholog of CCR5. MOP020 does not correspond to any of the three mouse chemokine receptor genes cloned recently [16], demonstrating the existence of a fourth murine chemokine receptor.

[0066] The sequence of CCR5 revealed a single open reading frame of 352 codons encoding a protein of 40,600 Da. The sequence surrounding the proposed initiation codon is in agreement with the consensus as described by Kozak [22], since the nucleotide in -3 is a purine. The hydropathy profile of the deduced amino acid sequence is consistent with the existence of 7 transmembrane segments. Alignment of the CCR5 amino acid sequence with that

of other functionally characterised human CC-chemokine receptors is represented in figure 2. The highest similarity is found with the CCR2 receptor [8] that shares 75.8 % identical residues. There is also 56.3 % identity with the CCR1 receptor [31], 58.4 % with the CCR3 [10], and 49.1% with the CCR4 [37]. CCR5 represents therefore a new member of the CC-chemokine receptor group [30]. Like the related CCR1 and IL-8 receptors [20, 29, 31, 16] the coding region of CCR5 appears as intronless. From our partial sequencing data, the CCR2 gene is also devoid of intron in the first two thirds of its coding sequence.

[0067] Sequence similarities within the chemokine receptor family are higher in the transmembrane-spanning domains, and in intracellular loops. As an example, the identity score between CCR5 and CCR2 goes un to 92% when considering the transmembrane segments only. Lower similarities are found in the N-terminal extracellular domain, and in the extracellular loops . The N-terminal domain of the IL-8 and CCR2 receptors has been shown to be essential for interaction with the ligand [19, 18]. The variability of this region among CC-chemokine receptors presumably contributes to the specificity towards the various ligands of the family.

[0068] A single potential site for N-linked glycosylation was identified in the third extracellular loop of CCR5 (figure 1). No glycosylation site was found in the N-terminal domain of the receptor, where most G protein-coupled receptors are glycosylated. The other chemokine receptors CCR1 and CCR2 present such an N-linked glycosylation site in their N-terminal domain [31, 8]. By contrast, the CCR3 receptor [10] does not display glycosylation sites neither in the N-terminus, nor in extracellular loops. The active CCR5 receptor has four cysteines in its extracellular segments, and all four are conserved in the other CC- and CXC-chemokine receptors (figure 2). The cysteines located in the first and second extracellular loops are present in most G protein-coupled receptors, and are believed to form a disulphide bridge stabilising the receptor structure [41]. The two other cysteines, in the N-terminal segment, and in the third extracellular loop could similarly form a stabilising bridge specific to the chemokine receptor family. The intracellular domains of CCR5 do not include potential sites for phosphorylation by protein kinase C (PKC) or protein kinase A. PKC sites, involved in heterologous desensitisation are frequent in the third intracellular loop and C-terminus of G protein-coupled receptors. CCR1 is also devoid of PKC sites. In contrast, all CC-chemokine receptors, are rich in serine and threonine residues in the C-terminal domain. These residues represent potential phosphorylation sites by the family of G protein-coupled receptor kinases, and are probably involved in homologous desensitisation [41]. Five of these S/T residues are perfectly aligned in all five receptors (figure 2).

Physical linkage of the CCR5 and CCR2 genes

[0069] As stated above, the 16 clones isolated with the MOP020 probe corresponded to a single contig containing the CCR5 and CCR2 genes. The organisation of this contig was investigated in order to characterise the physical linkage of the two receptor genes in the human genome. A combination of restriction mapping, Southern blotting, fragment subcloning and partial sequencing allowed to determine the respective borders and overlaps of all clones. Out of the 16 clones, 9 turned out to be characterised by a specific restriction map, and their organisation is depicted in figure 3. Four of these clones (#11, 18, 21, 22) contained the CCR2 gene alone, four clones (# 7, 13, 15, 16) contained the ChemR13 gene alone and one clone (#9) contains part of both coding sequences. The CCR2 and CCR5 genes are organised in tandem, CCR5 being located downstream of CCR2. The distance separating CCR2 and CCR5 open reading frames is 17.5 kb. The chromosomal localisation of the tandem is presently unknown. Other chemokine receptors have however been located in the human genome: the CCR1 gene was localised by fluorescence in situ hybridisation to the p21 region of human chromosome 3 [16]. The two IL-8 receptor genes, and their pseudogene have been shown to be clustered on the human 2q34-q35 region [1].

Functional expression and pharmacology of the active CCR5 receptor

[0070] Stable CHO-K1 cell lines expressing the active CCR5 receptor were established and were screened on the basis of the level of CCR5 transcripts as determined by Northern blotting. Three clones were selected and tested for biological responses in a microphysiometer, using various CC- and CXC-chemokines as potential agonists. Wild type CHO-K1 cells were used as control to ensure that the observed responses were specific for the transfected receptor, and did not result from the activation of endogenous receptors. The microphysiometer allows the real time detection of receptor activation, by measuring the modifications of cell metabolism resulting from the stimulation of intracellular cascades [33]. Several studies have already demonstrated the potential of microphysiometry in the field of chemokine receptors. Modifications of metabolic activity in human monocytes, in response CC-chemokines, were monitored using this system [43]. Similarly, changes in the acidification rate of THP-1 cells (a human monocytic cell line) in response to MCP-1 and MCP-3 have been measured [36]. The estimation of the EC_{50} for both proteins, using this procedure, was in agreement with the values obtained by monitoring the intracellular calcium in other studies [8, 15].

[0071] Ligands belonging to the CC- and CXC-chemokine classes were tested on the CCR5 transfected CHO-K1 cells. Whereas MIP-1α, MIP-1β and RANTES were found to be potent activators of the new receptor (figure 4), the

CC-chemokines MCP-1, MCP-2 and MCP-3, and the CXC-chemokines GROα and IL-8 had no effect on the metabolic activity, even at the highest concentrations tested (30 nM). The biological activity of one of the chemokines inducing no response on CCR5 (IL-8) could be demonstrated on a CHO-K1 cell line transfected with the IL-8A interleukin receptor (Mollereau et al., 1993) : IL-8 produced a 160 % increase in metabolic activity as determined using the microphysiometer. The biological activity of the MCP-2 and MCP-3 preparations as provided by J. Van Damme have been widely documented [2, 40]. MIP-1α, MIP-1β and RANTES were tested on the wild type CHO-K1 cells, at a 30 nM-concentration, and none of them induced a metabolic response. On the CCR5 transfected CHO-K1 cell line, all three active ligands (MIP-1α, MIP-1β and RANTES) caused a rapid increase in acidification rate, reaching a maximum by the second or third minute after perfusion of the ligand. The acidification rate returned to basal level within 10 minutes. The timing of the cellular response is similar to that observed for chemokines on their natural receptors in human monocytes [43]. When agonists were applied repeatedly to the same cells, the response was strongly reduced as compared to the first stimulation, suggesting the desensitisation of the receptor. All measurements were therefore obtained on the first stimulation of each capsule.

[0072] The concentration-effect relation was evaluated for the three active ligands in the 0.3 to 30 nM range (figure 3B and C). The rank order of potency was MIP-1α > MIP-1β = RANTES. At 30 nM concentrations, the effect of MIP-1α appeared to saturate (at 156 % of baseline level) while MIP-1β and RANTES were still in the ascending phase. Higher concentrations of chemokines could however not be used. The EC50 was estimated around 3 nM for MIP-1α. The concentrations necessary for obtaining a biological response as determined by using the microphysiometer are in the same range as those measured by intracellular calcium mobilisation for the CCR1 [31], the CCR2A and B [8], and the CCR3 [10] receptors. The ligand specificity of CCR5 is similar to that reported for CCR3 [10]. CCR3 was described as the first cloned receptor responding to MIP-1β. However, MIP-1β at 10 nM elicits a significant effect on the CCR5, while the same concentration is without effect on the CCR3 transfected cells [10]. These data suggest that CCR5 could be a physiological receptor for MIP-1β.

[0073] Binding experiments using [^{125}I]-human MIP-1α as ligand did not allow to demonstrate specific binding to CCR53 expressing CHO-K1 cells, using as much as 0.4 nM radioligand and 1 million transfected cells per tube. Failure to obtain binding data could be attributed to a relatively low affinity of the receptor for MIP-1α.

Northern blotting analysis

[0074] Northern blotting performed on a panel of dog tissues did not allow to detect transcripts for CCR5. Given the role of the chemokine receptor family in mediating chemoattraction and activation of various classes of cells involved in inflammatory and immune responses, the probe was also used to detect specific transcripts in a panel of human cell lines of haematopoietic origin (figure 5). The panel included lymphoblastic (Raji) and T lymphoblastic (Jurkat) cell lines, promyeloblastic (KG-1A) and promyelocytic (HL-60) cell lines, a monocytic (THP-1) cell line, an erythroleukemia (HEL 92.1.7) cell line, a megakaryoblastic (MEG-01) cell line, and a myelogenous leukaemia (K-562) cell line. Human peripheral blood mononuclear cells (PBMC), including mature monocytes and lymphocytes, were also tested. CCR5 transcripts (4.4 kb) could be detected only in the KG-1A promyeloblastic cell line, but were not found in the promyelocytic cell line HL-60, in PBMC, or in any of the other cell lines tested. These results suggest that the active CCR5 receptor could be expressed in precursors of the granulocytic lineage. CC-chemokines have been reported to stimulate mature granulocytes [27, 38, 23, 2]. However, recent data have also demonstrated a role of CC- and CXC-chemokines in the regulation of mouse and human myeloid progenitor cell proliferation [6, 7].

[0075] CCR5 was shown to respond to MIP-1α, MIP-1β and RANTES, the three chemokines identified as the major HIV-suppressive factors produced by CD8$^+$ T cells [9], and released in higher amounts by CD4$^+$ T lymphocytes from uninfected but multiply exposed individuals [51]. CCR5 represents a major co-receptor for macrophage-tropic (M-tropic) HIV-1 primary isolates and strains [45, 50]. M-tropic strains predominate during the asymptomatic phase of the disease in infected individuals, and are considered as responsible for HIV-1 transmission. Strains adapced for growth in transformed T-cell lines (T-tropic strains) use as a co-receptor LESTR (or fusin) [50], an orphan receptor also belonging to the chemokine receptor family, but not yet characterised functionally [21, 52, 53]. Dual-tropic viruses, which may represent transitional forms of the virus in late stages of infection [54] are shown to use both CCR5 and LESTR as co-receptors, as well as the CC-chemokine receptors CCR2b and CCR3 [47]. The broad spectrum of co-receptor usage of dual-tropic viruses suggests that within infected individuals, the virus may evolve at least in part from selection by a variety of co-receptors expressed on different cell types.

Identification of an inactive ΔCCR5 receptor

[0076] It is known that some individuals remain uninfected despite repeated exposure to HIV-1 [55, 56, 51]. A proportion of these exposed-uninfected individuals results from the relatively low risk of contamination after a single contact with the virus, but it has been postulated that truly resistant individuals do exist. In fact, CD4$^+$ lymphocytes isolated

from exposed-uninfected individuals are highly resistant to infection by primary M-tropic, but not T-tropic HIV-1 strains. Also, peripheral blood mononuclear cells (PBMC) from different donors are not infected equally with various HIV-1 strains [57-59]. Given the key role played by CCR5 in the fusion event that mediates infection by M-tropic viruses, it is postulated that variants of CCR5 could be responsible for the relative or absolute resistance to HIV-1 infection exhibited by some individuals, and possibly for the variability of disease progression in infected patients [66]. The Inventors selected three HIV-1 infected patients known to be slow progressors, and four seronegative individuals as controls; the full coding region of their CCR5 gene was amplified by PCR and sequenced. Unexpectedly, one of the slow progressors, but also two of the uninfected controls, exhibited heterozygosity at the CCR5 locus for a biallelic polymorphism. The frequent allele corresponded to the published CCR5 sequence, while the minor one displayed a 32 bp deletion within the coding sequence, in a region corresponding to the second extracellular loop of che receptor (Fig. 6). The figure 6 is the structure of the mutant form of human CC-chemokine receptor 5. *a*, The amino acid sequence of the non-functional Δccr5 protein is represented. The transmembrane organisation is given by analogy with the predicted transmembrane structure of the wild-type CCR5. Amino acids represented in black correspond to unnatural residues resulting from the frame shift caused by the deletion. The mutant protein lacks the lasc three transmembrane segments of CCR5, as well as the regions involved in G protein-coupling. *b*, Nucleotide sequence of the CCR5 gene surrounding the deleted region, and translation into the normal receptor (top) or the truncated mutant (ccr5, bottom). The 10-bp direct repeat is represented in italics. The full size coding region of the CCR5 gene was amplified by PCR, using 5'-TCGAGGATCCAAGATGGATTATCAAGT-3' and 5'-CTGATCTAGAGCCATGTGCACAACTCT-3' as forward and reverse primers respectively. The PCR products were sequenced on both strands using the same oligonucleotides as primers, as well as internal primers, and fluorochrome-labelled dideoxynucleotides as terminators. The sequencing products were run on an Applied Biosystem sequencer, and ambiguous positions were searched along the coding sequence. When the presence of a deletion was suspected from direct sequencing, the PCR products were cloned after restriction with *BamHI* and *XbaI* endonucleases into pcDNA3. Several clones were sequenced to confirm the deletion. The deletion was identical in three unrelated individuals investigated by sequencing.

[0077] Cloning of the PCR product and sequencing of several clones confirmed the deletion. The deletion causes a frame shift, which is expected to result in premature termination of translation. The protein encoded by this mutant allele (Δccr5) therefore lacks the last three transmembrane segments of the receptor. A 10-bp direct repeat flanking the deleted region (Fig. 6b) on both sides is expected to have promoted the recombination event leading to the deletion. Numerous mutagenesis studies performed on various classes of G protein-coupled receptors, including chemokine receptors, makes it clear that such a truncated protein is certainly not functional in terms of chemokine-induced signal transduction: it lacks the third intracellular loop and C-terminal cytoplasmic domains, the two regions involved primarily in G protein coupling [41]. In order to test whether the truncated protein was able to function as a HIV-1 co-receptor, the Inventors tested its ability to support membrane fusion by both primary M-tropic and dual-tropic virus ENV proteins. The recombinant protein was expressed in quail QT6 cells together with human CD4. The QT6 cells were then mixed with HeLa cells expressing the indicated viral ENV protein and the extent of cell-cell fusion measured using a sensitive and quantitative gene-reporter assay. In contrast to wild-type CCR5, the truncated receptor did not allow fusion with cells expressing the ENV protein from either M-tropic or dual-tropic viruses (Figure 7). The figure 7 represents the quantification of ENV protein-mediated fusion by luciferase assay. To quantify cell-cell fusion events, Japanese quail QT6 fibrosarcoma cells were transfected or cotransfected as indicated with the pcDNA3 vector (Invitrogen) containing the coding sequence for wild-type CCR5, the truncated ccr5 mutant, the CCR2b or the Duffy chemokine receptors, or with the pCDNA3 vector alone. The target cells were also transfected with human CD4 expressed from the CMV promoter and the luciferase gene under the control of the T7 promoter. HeLa effector cells were infected (MOI = 10) with vaccinia vectors expressing T7-polymerase (vTF1.1) and either the JR-FL (vCB28) or 89.6 (vBD3) envelope proteins. The luciferase activity resulting from cell fusion is expressed as the percentage of the activity (in relative light units) obtained for wild-type CCR5. All transfections were performed with an identical quantity of plasmid DNA using pcDNA3 as carrier when necessary. To initiate fusion, target and effector cells were mixed in 24 well plates at 37 °C in the presence of ara-C and rifampicin, and allowed to fuse for 8 hours. Cells were lysed in 150 μl of reporter lysis buffer (Promega) and assayed for luciferase activity according to the manufacturer's instructions (Promega).

[0078] Coexpression of Δccr5 with wild-type CCR5 consistently reduced the efficiency of fusion for both JR-FL and 89.6 envelopes, as compared with CCR5 alone. Whether this *in vitro* inhibitory effect (not shared by the chemokine receptor Duffy, used as control) also occurs *in vivo* is presently not known. Coexpression with the CCR2b receptor [31], which is the CC-chemokine receptor most closely related to CCR5 but does not promote fusion by M-tropic HIV-1 strains [48], did not rescue the mutation by formation of a hybrid molecule (Fig. 7).

[0079] The figure 8 represents genotyping of individuals by PCR and segregation of the CCR5 alleles in CEPH families. a, Autoradiography illustrating the pattern resulting from PCR amplification and *EcoRI* cleavage for individuals homozygous for the wild-type CCR5 allele (CCR5/CCR5), the null Δccr5 allele (Δccr5/Δccr5), and for heterozygotes (CCR5/Δccr5). A 735 bp PCR product is cleaved into a common band of 332 bp for both alleles, and into 403 and 371 bp bands for the wild-type and mutant alleles, respectively, *b*, Segregation of the CCR5 alleles in two informative

families of the CEPH. Half-black and white symbols represent heterozygotes and wild-type homozygotes, respectively. For a few individuals in the pedigrees, DNA was not available (ND: not determined). PCRs were performed on genomic DNA samples, using 5'-CCTGGCTGTCGTCCATGCTG-3' and 5'-CTGATCTAGAGCCATGTGCACAACTCT-3' as forward and reverse primers respectively. Reaction mixtures consisted in 30 µl of 10 mM Tris-HCl buffer pH 8.0, containing 50 mM Kcl, 0.75 mM $MgCl_2$, 0.2 mM dCTP, dGTP and dTTP, 0.1 mM dATP, 0.5 µi [α-^{32}P]-dATP, 0.01% gelatine, 5% DMSO, 200 ng target DNA, 60 ng of each of the primers and 1.5 U Taq polymerase. PCR conditions were: 93 °C for 2 min 30; 93 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min, 30 cycles; 72 °C for 6 min. After the PCR reaction, the samples were incubated for 60 min at 37 °C with 10 U *Eco*RI, and 2 µl of the denatured reaction mixture was applied onto a denaturing 5% polyacrylamide gel containing 35% formamide and 5.6 M urea. Bands were detected by autoradiography.

[0080] Based on the 14 chromosomes tested in the first experiment, the deleted Δccr5 allele appeared rather frequent in the Caucasian population. The accurate frequency was further estimated by testing (Fig. 8a) a large cohort of Caucasian individuals, including unrelated members of the CEPH (Centre d'Etude des Polymorphismes Humains) families, part of the IRIBHN staff, and a bank of anonymous DNA samples from healthy individuals collected by the Genetics Department of the Erasme Hospital in Brussels. From a total of more than 700 healthy individuals, the allele frequencies were found to be 0.908 for the wild-type allele, and 0.092 for the mutant allele (Table I). The genotype frequencies observed in the population were not significantly different from the expected Hardy-Weinberg distribution (CCR5/CCR5: 0.827 vs 0.824; CCR5/Δccr5: 0.162 vs 0.167; Δccr5/Δccr5: 0.011 vs 0.008, $p > 0.999$), suggesting that the null allele has no drastic effect on fitness. Using two informative CEPH families, it was confirmed that the wild-type CCR5 gene and its Δccr5 variant were allelic, and segregated in a normal mendelian fashion (Fig. 8b). Interestingly, a cohort of 124 DNA samples originating from Central Africa (collected from Zaire, Burkina Fasso, Cameroun, Senegal and Benin) and Japan did not reveal a single Δccr5 mutant allele, suggesting that this allele is either absent or very rare in Asian, African black populations (Table I).

[0081] The consequences of the existence of a null allele of CCR5 in the normal Caucasian population were then considered in terms of susceptibility to infection by HIV-1. If, as it is predicted, CCR5 plays a major (not redundant) role in the entry of most primary virus strains into cells, then Δccr5/Δccr5 individuals should be particularly resistant to HIV-1 challenge, both *in vitro* and *in vivo*. The frequency of the Δccr5/Δccr5 genotype should therefore be significantly lower in HIV-1 infected patients, and increased in exposed-uninfected individuals. Also, if hecerozygotes have a statistical advantage due to the lower number of functional receptors on their white blood cells, or to the possible dominant-negative properties of the mutant allele, the frequency of heterozygotes (and mutant alleles) should be decreased in HIV-infected populations. These hypotheses were tested by genocyping a large number of seropositive Caucasian individuals ($n = 645$) belonging to cohorts originating from various hospitals from Brussels, Liège and Paris (Table I). Indeed, it was found that within this large series, the frequency of the null Δccr5 allele was significantly reduced from 0.092 to 0.053 ($p < 10^{-5}$). The frequency of heterozygotes was also reduced from 0.162 to 0.106 ($p < 0.001$) and not a single Δccr5/Δccr5 individual could be found ($p < 0.01$).

[0082] Altogether, functional and statistical data suggest that CCR5 is indeed the major co-receptor responsible for natural infection by M-tropic HIV-1 strains. Individuals homozygous for the null Δccr5 allele (about 1% of the Caucasian population) have apparently a strong resistance to infection. It is unclear at this point whether resistance to HIV-1 is absolute or relative, and whether resistance will vary depending on the mode of viral contamination. Larger cohorts of seropositive individuals will have to be tested in order to clarify this point. Heterozygotes have a milder though significant advantage: assuming an equal probability of contact with HIV, it can be inferred from Table I that heterozygotes have a 39% reduction in their likeliness of becoming seropositive, as compared to individuals homozygous for the wild-type CCR5 allele. Both a decrease in functional CCR5 receptor number, and a dominant-negative effect of Δccr5 *in vivo*, comparable to what is observed in the *in vitro* experiments (Fig. 7) are possible explanations for this relative protection. The mutant allele, which can be regarded as a natural knock-out in human, is not accompanied by an obvious phenotype in homozygous individuals. Nevertheless, the lack of overt phenotype, taken together with the relative protection that characterises heterozygous subjects, suggests that pharmacological agents that selectively block the ability of HIV-1 to utilise CCR5 as a cofactor, could be effective in preventing HIV-1 infection, and would be predicted not be associated with major side effects resulting from CCR5 inactivation. These pharmaceutical agents could be used with other compounds which are able to block other chemokine receptors used as co-receptors by some HIV-primary isolates in order to infect other cells [47]. The prevalence of the null allele in the Caucasian population raises the question of whether pandemia of HIV (or related viruses using the same co-receptor) have contributed during mankind's evolution to stabilise by selection the mutant ccr5 allele at such a high frequency.

Production of antibodies anti-CCR5

[0083] Antibodies were produced by genetic immunisation. Six week old females balb/c mice were used. DNA coding for the human CCR5 receptor was inserted in the expression vector pcDNA3 under the control of the CMV promotor

and 100 µg DNA was injected in the anterior tibial muscle, five days after pre-treatment of this muscle with cardiotoxine (from venom of Naja Nigricolis). Injections were repeated twice at three week intervals. Fifteen days after the last injection, blood was taken from each animal and sera were tested for the presence of anti-CCR5 antibodies.

Test of sera using Fluorescence Activatad Cell Sorter (FACS)

[0084] Sera were tested by fluorescence activated cell sorting using recombinant CHO cells expressing the CCR5 receptor. Briefly, cells were detached using a PBS-EDTA-EGTA solution and incubated into PBS-BSA medium for 30 minutes at room temperature with 5 µl serum on the basis of 100,000 cells per tube. Cells were then washed and incubated for 30 minutes in ice together with anti-mouse antibody labelled with fluorescein. Cells were washed, taken up into 200 µl of a PBS-BSA solution and fluorescence was analysed by FACS (FACSCAN, Becton-Dickinson). 10,000 cells were counted. Wild type CHO or recombinant CHO cells expressing the human CCR2b receptor were used as controls.

[0085] When tested by FACS analysis 2 weeks after the last injection (figure 9), all the sera from mice immunised with CCR5 cDNA, clearly recognised the native receptor expressed on CHO cells (mean of fluorescence = 200), without significant cross reaction with control cells expressing CCR2b (mean of fluorescence = 20).

[0086] Sera were tested on either a CHO cell line expressing high level of CCR5 receptor (black histogram) or a CHO cell line expressing CCR2b receptor (white histogram) as negative control. Each serum was tested individually.

Antibodies anti-CCR5 and HIV infectivity

[0087] Peripheral blood mononuclear cells (PBMC) from one donor homozygous from wild type CCR5 gene, were isolated and cultivated 3 days in presence of PHA.

[0088] On day 4, 800 µl of cells (10^5 cells/ml) were incubated with 8 µl of sera from mice immunised with CCR5 cDNA, 30 minutes at 37 °C. 1 ml of viral solution (JRCSF HIV strain) is then added and incubated during 2 hours. Cells were then washed twice and cultivated during 15 days.

[0089] Aliquot of medium is caken ac days 0, 4, 7, 10 and 14 and the dosage of antigen p24 is performed.

[0090] 14 days after the beginning of the experiment, one serum (serum B0) totally block the production of p24, indicating its ability to block the infection of the lymphocytes by this HIV strain (figure 10). Other serums also exhibit a partial or total effect on this infection (serum A2 and B1). All the other sera did not show any effect on this infection.

Production of monoclonal antibodies

[0091] Mice with the highest title of CCR5 antibodies were selected for monoclonal antibodies production and injected intravenously with 10^7 recombinant CHO-K1 cells expressing human CCR5 receptors. Three days later, animals were sacrificed and fusion of splenic cells or cells from lymph nodes near the site of injection with SP2/0 myeloma cells, were performed. Fusion protocol used was that of Galfre et al. (Nature 266, 550 (1977)). A selective HAT (hypoxanthine/aminopterin/thymidin) medium is used to select hybridomas and their supernatants are tested by FACS using recombinant CHO cells expressing the human CCR5 receptor, as it was done for the sera. Positives hybridomas are then cloned by limited dilution. Clones that are shown positive by FACS analyses are then expanded and produced in ascites in balb/C mice.

Table 1

	Seronegative			Seropositive			Chi-squared
	Number	Frequency	Standard error	Number	Frequency	Standard error	
Genotypes :							2 degrees of freedom 17.7 p < 0.0005
CCR5/CCR5	582	0.827	0.014	645	0.892	0.012	
CCR5/Δ CCR5	114	0.162	0.014	78	0.108	0.012	
CCR5/Λ CCR5	8	0.011	0.004	0	0.000	< 0.001	
Total :	704	1.000		723	1.000		
Alleles :							1 degree of freedom p < 0.0005
CCR5	1278	0.908	0.008	1368	0.946	0.006	
Λ CCR5	130	0.092	0.008	78	0.054	0.006	
Total :	1408	1.000		1446	1.000		

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[0092]

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Claims

1. A method for determining whether an anti-ligand is capable of inhibiting the binding of a ligand, said ligand being an HIV virus or a portion thereof, to a peptide having an amino acid sequence which presents more than 80% homology with SEQ ID NO. 2 shown in figure 1, said method comprising the steps of contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said peptide, with the anti-ligand under conditions permitting a binding of the anti-ligand to said peptide and determining whether the said anti-ligand inhibits the binding of the said ligand, to said peptide.

2. A method for determining whether an anti-ligand is capable of inhibiting the binding of a ligand, said ligand being an HIV virus or a portion thereof, to a peptide having an amino acid sequence which presents more than 80% homology with SEQ ID NO. 2 shown in figure 1, said method comprising the steps of:

   - preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said peptide,
   - isolating a membrane fraction from the cell extract,
   - contacting the anti-ligand with the membrane fraction under conditions permitting binding of the anti-ligand to said peptide, and
   - determining whether the said anti-ligand inhibits the binding of the said ligand to said peptide.

3. Method according to claim 1 or 2, wherein the method comprises the steps of:

   - preparing a cell or cell extract, said cell being transfected with the nucleic acid molecule encoding a peptide having an amino acid sequence which presents more than 80% homology with SEQ ID NO. 2 shown in figure 1,
   - possibly isolating a membrane fraction from the cell extract,
   - contacting the cell or membrane fraction with said anti-ligand, in the presence of the said ligand of said peptide, under conditions permitting the activation of a functional peptide response, and
   - detecting by means of a bio-assay a modification in the activity of the peptide, thereby determining whether the said anti-ligand inhibits the binding of the said ligand to said peptide.

4. A method of screening drugs to identify drugs which specifically bind the peptide comprising the amino acid sequence which presents more than 80% homology with SEQ ID NO. 2 shown in figure 1, or a part thereof, and can be used in the treatment and/or prevention of HIV virus infection, said method comprising contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said peptide, with a drug under conditions permitting binding of said drug to said peptide and determining whether said drug specifically binds the transfected cell, thereby identifying a drug which specifically binds to said peptide and which can be used in the treatment and/or prevention of HIV virus infection.

5. A method of screening drugs to identify drugs which specifically bind the peptide comprising the amino acid sequence which presents more than 80% homology with SEQ ID NO. 2 shown in figure 1, or a part thereof, and can be used in the treatment and/or prevention of HIV virus infection, said method comprising preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said peptide, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a drug under conditions permitting binding of said drug to said peptide and determining whether said drug specifically binds the transfected cell, thereby identifying a drug which specifically binds to said peptide and which can be used in the treatment and/or prevention of HIV virus infection.

6. A method for determining whether an agonist or antagonist which binds to a peptide comprising the amino acid sequence which presents more than 80% homology with SEQ ID NO. 2 shown in figure 1, or a part thereof, can be used for the treatment and/or prevention of an HIV virus infection, said method comprising contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said peptide with the agonist or antagonist under conditions permitting binding of the agonist or antagonist to said peptide and determining whether the said agonist or antagonist inhibits the binding of HIV virus to said peptide.

7. A method for determining whether an agonist or antagonist which binds to a peptide comprising the amino acid sequence which presents more than 80% homology with SEQ ID NO. 2 shown in figure 1, or a part thereof, can be used for the treatment and/or prevention of an HIV virus infection, said method comprising preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said peptide, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the agonist or antagonist under conditions permitting binding of the agonist or antagonist to said peptide and determining whether the said agonist or antagonist inhibits the binding of HIV virus to said peptide.

8. The method according to any of the claims 1 to 7, wherein said HIV virus is selected from the group consisting of the human immunodeficiency virus 1 (HIV 1), the human immunodeficiency virus 2 (HIV 2) or a portion of said HIV viruses.

9. The method according to any of the claims 1 to 8, wherein the portion of said HIV virus is the glycoprotein GP120/GP160 or a portion thereof.

10. The method according to any of the claims 1 to 9, further comprising the step of measuring the infectivity of the cell by an HIV strain wherein said anti-ligand, drug, agonist or antagonist decreases infectivity by said HIV strain.

11. The method according to claim 10, wherein the cell is a lymphocyte cell.

12. The method according to claim 10 or 11, wherein the HIV strain is the human immunodeficiency virus 1 (HIV-1).

13. The method according to claim 10 or 11, wherein the HIV strain is the human immunodeficiency virus 2 (HIV-2).

14. The method according to any of the claims 10 to 13, wherein the decrease in HIV infectivity is measured by the dosage of an HIV protein.

15. The method according to claim 14, wherein said HIV protein is the HIV antigen P24.

16. The method according to any of the claims 1 to 15, wherein the amino acid sequence of the peptide presents more than 90% homology with SEQ ID NO. 2 shown in figure 1.

17. The method according to any of the claims 1 to 15, wherein the amino acid sequence of the peptide presents more than 95% homology with SEQ ID NO. 2 shown in figure 1.

18. The method according to any of the claims 1 to 15, wherein the amino acid sequence of the peptide is the amino acid sequence SEQ ID NO. 2 shown in figure 1 or a portion thereof comprising at least the N-terminus segment and the first extracellular loop of the amino acid sequence SEQ ID NO. 2 shown in figure 1.

19. The method according to claim 3, wherein the modification in the peptide activity is detected by a bio-assay based upon the modification in a production of a second messenger.

20. The method of claim 19, wherein the bio-assay is based upon a measurement of calcium ions or inositol phosphates (such as IP_3) concentration.

21. The method according to any one of the claims 1 to 20, wherein the cell is a mammalian cell non neuronal in origin, preferably selected from the group consisting of CHO-K1, HEK293, BHK21 and COS-7 cells.

22. The method according to any of the claims 1 to 21, wherein the anti-ligand, the drug, the agonist or the antagonist is an antibody.

23. The method according to claim 22, wherein the antibody is a monoclonal antibody.

24. The method according to claim 23, wherein the monoclonal antibody is directed to an epitope of the said peptide present on the surface of a cell.

25. An antibody which inhibits or reduces the binding of the human immunodeficiency virus 1 (HIV 1) or the human immunodeficiency 2 (HIV 2) to a peptide having an amino acid sequence presenting more than 80% homology with SEQ ID NO. 2 shown in figure 1, identified according to the method of any of the claims 1 to 24.

26. The antibody according to the claim 25, wherein the amino acid sequence of the peptide presents more than 90% homology with SEQ ID NO. 2 shown in figure 1.

27. The antibody according to the claim 25, wherein the amino acid sequence of the peptide presents more than 95% homology with SEQ ID NO. 2 shown in figure 1.

28. The antibody according to the claim 25, wherein the amino acid sequence of the peptide is the amino acid sequence SEQ ID NO. 2 shown in figure 1.

29. The antibody according to any of the claims 25 to 28, which is a monoclonal antibody.

30. The antibody according to claim 29, which is a monoclonal antibody directed to an epitope of the said peptide, present on the surface of a cell expressing said peptide.

31. The antibody according to any of the claims 25 to 30, which decreases infectivity of a cell by an HIV strain.

32. The antibody according to claim 31, wherein the cell is a lymphocyte cell.

33. The antibody according to claim 31 or 32, wherein the HIV strain is a human immunodeficiency virus 1 (HIV-1 strain).

34. The antibody according to claim 31 or 32, wherein the HIV strain is a human immunodeficiency virus 2 (HIV-2 strain).

35. A pharmaceutical composition comprising an adequate pharmaceutical carrier and a sufficient amount of the antibody according to any of the claims 25 to 34.

36. The use of the pharmaceutical composition according to the claim 35 for the manufacture of a medicament in the prevention and/or the treatment of an infection by the human immunodeficiency virus 1 (HIV-1) and/or the human immunodeficiency virus 2 (HIV-2) (AI DS).

37. Use of an antisense oligonucleotide, comprising a sequence which specifically hybridizes to a nudeic acid molecule having more than 80% homology with nucleic acid sequence SEQ ID NO. 2 shown in figure 1, for the manufacture of a medicament in the prevention and/or the treatment of an infection by the human immunodeficiency virus 1 (HIV-1) and/or the human immunodeficiency virus 2 (HIV-2) (AIDS).

38. Use of an antisense oligonucleotide, comprising a sequence which specifically hybridizes to a nucleic acid molecule having at least the nucleic acid sequence SEQ ID NO. 2 shown in figure 1 or a portion thereof, for the manufacture of a medicament in the prevention and/or the treatment of an infection by the human immunodeficiency virus 1 (HIV-1) and/or the human immunodeficiency virus 2 (HIV-2) (AIDS).

39. The use according to the claim 37 or 38, wherein said nucleic acid molecule encodes a peptide as defined in any of the claims 1 to 7 and 16 to 18.

40. The use according to any of the claims 37 to 39, wherein said nucleic acid molecule is a cDNA molecule or a genomic DNA molecule.

41. The use according to any of the claims 37 to 40, wherein said antisense oligonucleotide comprises chemical analogs of nucleotides.

Patentansprüche

1. Verfahren zur Bestimmung, ob ein Anti-Ligand in der Lage ist, die Bindung eines Liganden an ein Peptid mit einer Aminosäure-Sequenz, die mehr als 80% Homologie mit der Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, aufweist, zu inhibieren, wobei der Ligand ein HIV-Virus oder ein Teil dessen ist, und wobei das Verfahren die Schritte umfasst, eine Zelle, die mit einem Vektor transfiziert ist, der das Nukleinsäure-Molekül, das für das Peptid kodiert, exprimiert, mit dem Anti-Liganden unter Bedingungen in Kontakt zu bringen, die die Bindung des Anti-Liganden an das Peptid erlauben, und zu bestimmen, ob der Anti-Ligand die Bindung des Liganden an das Peptid inhibiert.

2. Verfahren zur Bestimmung, ob ein Anti-Ligand in der Lage ist, die Bindung eines Liganden an ein Peptid mit einer Aminosäure-Sequenz, die mehr als 80% Homologie mit der Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, aufweist, zu inhibieren, wobei der Ligand ein HIV-Virus oder ein Teil dessen ist, und wobei das Verfahren die Schritte umfasst,

   - einen Zellextrakt von Zellen zuzubereiten, die mit einem Vektor transfiziert worden sind, der das Nukleinsäure-Molekül, das für das Peptid kodiert, exprimiert,
   - aus diesem Zellextrakt eine Membran-Fraktion zu isolieren,
   - den Anti-Liganden mit der Membran-Fraktion unter Bedingungen in Kontakt zu bringen, die die Bindung des Anti-Liganden an das Peptid erlauben, und
   - zu bestimmen, ob der Anti-Ligand die Bindung des Liganden an das Peptid inhibiert.

3. Das Verfahren nach Anspruch 1 oder 2, wobei das Verfahren die Schritte umfasst:

   - eine Zelle oder einen Zellextrakt zuzubereiten, wobei die Zelle mit dem Nukleinsäure-Molekül transfiziert worden ist, das für ein Peptid kodiert, das eine Aminosäure-Sequenz aufweist, die mehr als 80% Homologie mit der Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, aufweist,
   - gegebenenfalls eine Membran-Fraktion aus dem Zellextrakt zu isolieren,
   - die Zelle oder Membran-Fraktion in Gegenwart des Liganden des besagten Peptids mit dem Anti-Liganden unter Bedingungen in Kontakt zu bringen, die die Aktivierung einer funktionellen Peptid-Antwort erlauben, und
   - mittels eines Bioassays eine Modifikation in der Aktivität des Peptids nachzuweisen und dadurch zu ermitteln, ob der Anti-Ligand die Bindung des Liganden an das Peptid inhibiert.

4. Verfahren zum Durchmustern von Wirkstoffen zur Identifizierung von Wirkstoffen, die spezifisch an das Peptid binden, das eine Aminosäure-Sequenz aufweist, die mehr als 80% Homologie aufweist mit der Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, oder einen Teil von dieser aufweist, und für die Behandlung und/oder Vermeidung von HIV-Virus-Infektionen verwendet werden können, wobei das Verfahren die Schritte umfasst, eine Zelle, die mit einem Vektor transfiziert worden ist, der das Nukleinsäure-Molekül exprimiert, das für das Peptid kodiert, mit einem Wirkstoff unter Bedingungen in Kontakt zu bringen, die die Bindung des Wirkstoffs an das Peptid erlauben, und zu bestimmen, ob der Wirkstoff die transfizierte Zelle spezifisch bindet, um dadurch einen Wirkstoff zu identifizieren, der das Peptid spezifisch bindet und der für die Behandlung und/oder Vermeidung von HIV-Virus-Infektionen verwendet werden kann.

5. Verfahren zum Durchmustern von Wirkstoffen zur Identifizierung von Wirkstoffen, die spezifisch an das Peptid binden, das eine Aminosäure-Sequenz aufweist, die mehr als 80% Homologie aufweist mit der Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, oder einen Teil von dieser aufweist, und für die Behandlung und/oder Vermeidung von HIV-Virus-Infektionen verwendet werden können, wobei das Verfahren die Schritte umfasst, einen Zellextrakt aus Zellen zuzubereiten, die mit einem Vektor transfiziert worden sind, der das Nukleinsäure-Molekül exprimiert, das für das Peptid kodiert, aus dem Zellextrakt eine Membran-Fraktion zu isolieren, die Membran-Fraktion mit einem Wirkstoff unter Bedingungen in Kontakt zu bringen, die die Bindung des Wirkstoffs an das Peptid erlauben, und zu bestimmen, ob der Wirkstoff die transfizierte Zelle spezifisch bindet, um dadurch einen Wirkstoff zu identifizieren, der das Peptid spezifisch bindet und für die Behandlung und/oder Vermeidung von HIV-Virus-Infektionen verwendet werden kann.

6. Verfahren zur Bestimmung, ob ein Agonist oder Antagonist, der ein Peptid bindet, das eine Aminosäure-Sequenz umfasst, die mehr als 80% Homologie aufweist mit der Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, oder einen Teil von dieser aufweist, für die Behandlung und/oder Vermeidung einer HIV-Virus-Infektion verwendet werden kann, wobei das Verfahren die Schritte umfasst, eine Zelle, die mit einem Vektor transfiziert worden ist, der

das Nukleinsäure-Molekül exprimiert, das für das Peptid kodiert, mit dem Agonisten oder Antagonisten unter Bedingungen in Kontakt zu bringen, die die Bindung des Agonisten oder Antagonisten an das Peptid erlauben, und zu bestimmen, ob der Agonist oder Antagonist die Bindung von HIV-Virus an das Peptid inhibiert.

7. Verfahren zur Bestimmung, ob ein Agonist oder Antagonist, der ein Peptid bindet, das eine Aminosäure-Sequenz umfasst, die mehr als 80% Homologie aufweist mit der Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, oder einen Teil von dieser aufweist, für die Behandlung und/oder Vermeidung einer HIV-Virus-Infektion verwendet werden kann, wobei das Verfahren die Schritte umfasst, einen Zellextrakt aus Zellen zuzubereiten, die mit einem Vektor transfiziert worden sind, der das Nukleinsäure-Molekül exprimiert, das für das Peptid kodiert, aus dem Zellextrakt eine Membran-Fraktion zu isolieren, die Membran-Fraktion mit dem Agonisten oder Antagonisten unter Bedingungen in Kontakt zu bringen, die die Bindung des Agonisten oder Antagonisten an das Peptid erlauben, und zu bestimmen, ob der Agonist oder Antagonist die Bindung von HIV-Virus an das Peptid inhibiert.

8. Das Verfahren nach einem der Ansprüche 1 bis 7, wobei der HIV-Virus ausgewählt ist aus HIV-1, HIV-2 oder Teilen dieser HIV-Viren.

9. Das Verfahren nach einem der Ansprüche 1 bis 8, wobei der Teil des HIV-Virus das Glykoprotein gp120/gp160, oder ein Teil von diesen, ist.

10. Das Verfahren nach einem der Ansprüche 1 bis 9, wobei das Verfahren weiterhin den Schritt umfasst, den Grad der Infektion der Zelle durch einen HIV-Stamm zu bestimmen, wobei der Anti-Ligand, der Wirkstoff, der Agonist oder Antagonist die Infektion durch den HIV-Stamm abschwächt.

11. Das Verfahren nach Anspruch 10, wobei die Zelle ein Lymphozyt ist.

12. Das Verfahren nach Anspruch 10 oder 11, wobei der HIV-Stamm das HIV-1 ist.

13. Das Verfahren nach Anspruch 10 oder 11, wobei der HIV-Stamm HIV-2 ist.

14. Das Verfahren nach einem der Ansprüche 10 bis 13, wobei die Abschwächung der HIV-Infektion gemessen wird über die Konzentration eines HIV-Proteins.

15. Das Verfahren nach Anspruch 14, wobei das HIV-Protein das HIV-Antigen p24 ist.

16. Das Verfahren nach einem der Ansprüche 1 bis 15, wobei die Aminosäure-Sequenz des Peptids mehr als 90% Homologie mit der Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, aufweist.

17. Das Verfahren nach einem der Ansprüche 1 bis 15, wobei die Aminosäure-Sequenz des Peptids mehr als 95% Homologie mit der Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, aufweist.

18. Das Verfahren nach einem der Ansprüche 1 bis 15, wobei die Aminosäure-Sequenz des Peptids die Aminosäure-Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, oder einen Teil von dieser, der wenigstens das N-terminale Segment und den ersten extrazellulären Loop der Aminosäure-Sequenz SEQ ID NO:2, dargestellt in Figur 1, umfasst, ist.

19. Das Verfahren nach Anspruch 3, wobei die Modifikation der Peptid-Aktivität mittels eines Bioassays auf Basis der Modifikation in der Produktion eines sekundären Botenstoffs nachgewiesen wird.

20. Das Verfahren nach Anspruch 19, wobei der Bioassay auf einer Messung der Konzentration der Calcium-Ionen oder von Inositol-Phosphaten (wie IP_3) beruht.

21. Das Verfahren nach einem der Ansprüche 1 bis 20, wobei die Zelle eine Säugetier-Zelle nicht-neuronalen Ursprungs ist, vorzugsweise ausgewählt aus CHO-K1-, HEK293-, BHK21- und COS-7-Zellen.

22. Das Verfahren nach einem der Ansprüche 1 bis 21, wobei der Anti-Ligand, der Wirkstoff, der Agonist oder der Antagonist ein Antikörper ist.

23. Das Verfahren nach Anspruch 22, wobei der Antikörper ein monoklonaler Antikörper ist.

24. Das Verfahren nach Anspruch 23, wobei der monoklonale Antikörper auf ein Epitop des Peptids gerichtet ist, das auf der Oberfläche einer Zelle vorliegt.

25. Antikörper, der die Bindung von HIV-1 oder von HIV-2 an ein Peptid mit einer Aminosäure-Sequenz, die mehr als 80% Homologie mit der Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, aufweist, inhibiert oder reduziert, und der identifiziert wurde nach dem Verfahren nach einem der Ansprüche 1 bis 24.

26. Der Antikörper nach Anspruch 25, wobei die Aminosäure-Sequenz des Peptids mehr als 90% Homologie mit der Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, aufweist.

27. Der Antikörper nach Anspruch 25, wobei die Aminosäure-Sequenz des Peptids mehr als 95% Homologie mit der Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, aufweist.

28. Der Antikörper nach Anspruch 25, wobei die Aminosäure-Sequenz des Peptids die Aminosäure-Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, ist.

29. Der Antikörper nach einem der Ansprüche 25 bis 28, der ein monoklonaler Antikörper ist.

30. Der Antikörper nach Anspruch 29, der ein monoklonaler Antikörper ist, der auf ein Epitop des Peptids gerichtet ist, das auf der Oberfläche einer dieses Peptid exprimierenden Zelle sitzt.

31. Der Antikörper nach einem der Ansprüche 25 bis 30, wobei der Antikörper den Grad der Infektion einer Zelle durch einen HIV-Stamm vermindert.

32. Der Antikörper nach Anspruch 31, wobei die Zelle ein Lymphozyt ist.

33. Der Antikörper nach Anspruch 31 oder 32, wobei der HIV-Stamm ein HIV-1-Stamm ist.

34. Der Antikörper nach Anspruch 31 oder 32, wobei der HIV-Stamm ein HIV-2-Stamm ist.

35. Pharmazeutische Zusammensetzung umfassend einen geeigneten pharmazeutischen Träger und eine ausreichende Menge des Antikörpers nach einem der Ansprüche 25 bis 34.

36. Verwendung der pharmazeutischen Zusammensetzung nach Anspruch 35 für die Herstellung eines Medikaments für die Prophylaxe und/oder Behandlung einer Infektion durch HIV-1 und/oder HIV-2 (AIDS).

37. Verwendung eines Antisense-Oligonukleotids umfassend eine Sequenz, die spezifisch mit einem Nukleinsäure-Molekül hybridisiert, das mehr als 80% Homologie mit der Nukleinsäure-Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, aufweist, für die Herstellung eines Medikaments für die Prophylaxe und/oder die Behandlung einer Infektion durch HIV-1 und/oder HIV-2 (AIDS).

38. Verwendung eines Antisense-Oligonukleotids umfassend eine Sequenz, die spezifisch mit einem Nukleinsäure-Molekül hybridisiert, das zumindest die Nukleinsäure-Sequenz SEQ ID NO:2, wie in Figur 1 dargestellt, oder einen Teil von dieser Sequenz aufweist, für die Herstellung eines Medikaments für die Prophylaxe und/oder Behandlung einer Infektion durch HIV-1 und/oder HIV-2 (AIDS).

39. Verwendung nach Anspruch 37 oder 38, wobei das Nukleinsäure-Molekül für ein Peptid kodiert, wie es in einem der Ansprüche 1 bis 7 und 16 bis 18 definiert ist.

40. Verwendung nach einem der Ansprüche 37 bis 39, wobei das Nukleinsäure-Molekül eine cDNA oder eine genomische DNA ist.

41. Verwendung nach einem der Ansprüche 37 bis 40, wobei das Antisense-Oligonukleotid chemische Analoga von Nukleotiden aufweist.

Revendications

1. Méthode pour déterminer si un anti-ligand est capable d'inhiber la liaison d'un ligand, ledit ligand étant un virus VIH ou une portion de celui-ci, à un peptide ayant une séquence en acides aminés qui présente plus de 80 % d'homologie avec SEQ ID NO. 2 représentée sur la figure 1, ladite méthode comprenant les étapes de mise en contact d'une cellule transfectée avec un vecteur exprimant la molécule d'acide nucléique codant ledit peptide, avec l'anti-ligand dans des conditions permettant une liaison de l'anti-ligand audit peptide et déterminant si l'anti-ligand inhibe la liaison dudit ligand, audit peptide.

2. Méthode pour déterminer si un anti-ligand est capable d'inhiber la liaison d'un ligand, ledit ligand étant un virus VIH ou une portion de celui-ci, à un peptide ayant une séquence en acides aminés qui présente plus de 80 % d'homologie avec SEQ ID NO. 2 représentée sur la figure 1, ladite méthode comprenant les étapes de :

   - préparation d'un extrait cellulaire à partir de cellules transfectées avec un vecteur exprimant la molécule d'acide nucléique codant ledit peptide,
   - isolation d'une fraction de membrane à partir de l'extrait cellulaire,
   - mise en contact de l'anti-ligand avec la fraction de membrane dans des conditions permettant la liaison de l'anti-ligand audit peptide, et
   - détermination de savoir si l'anti-ligand inhibe la liaison dudit ligand audit peptide

3. Méthode selon l'une des revendications 1 ou 2, dans laquelle la méthode comprend les étapes de :

   - préparation d'une cellule ou d'un extrait cellulaire, ladite cellule étant transfectée avec la molécule d'acide nucléique codant un peptide ayant une séquence en acides aminés qui présente plus de 80 % d'homologie avec SEQ ID NO. 2 représentée sur la figure 1,
   - éventuellement isolation d'une fraction de membrane à partir d'un extrait cellulaire,
   - mise en contact de la cellule ou de la fraction de membrane avec ledit anti- ligand, en présence du ligand dudit peptide, dans des conditions permettant l'activation d'une réponse peptidique fonctionnelle, et
   - détection au moyen d'un test biologique d'une modification dans l'activité du peptide, ce qui permet de déterminer si ledit anti-ligand inhibe la liaison dudit ligand audit peptide.

4. Méthode de criblage de médicaments pour identifier des médicaments qui se lient de façon spécifique au peptide ayant une séquence en acides aminés qui présente plus de 80 % d'homologie avec SEQ ID NO. 2 représentée sur la figure 1, ou une partie de celle-ci, et qui peut être utilisée dans le traitement et/ou la prévention de l'infection au virus VIH, ladite méthode comprenant la mise en contact d'une cellule transfectée avec un vecteur exprimant la molécule d'acide nucléique codant ledit peptide, avec un médicament dans des conditions permettant la liaison dudit médicament audit peptide et déterminant si le ledit médicament se lie de façon spécifique à la cellule transfectée, et ainsi identifiant un médicament qui se lie de façon spécifique audit peptide et qui peut être utilisé dans le traitement et/ou la prévention de l'infection au virus VIH.

5. Méthode de criblage de médicaments pour identifier des médicaments qui se lient de façon spécifique au peptide ayant une séquence en acides aminés qui présente plus de 80 % d'homologie avec SEQ ID NO. 2 représentée sur la figure 1, ou une partie de celle-ci, et qui peut être utilisée dans le traitement et/ou la prévention de l'infection au virus VIH, ladite méthode comprenant la préparation d'un extrait cellulaire à partir de cellules transfectées avec un vecteur exprimant la molécule d'acide nucléique codant ledit peptide, l'isolation d'une fraction de membrane à partir de l'extrait cellulaire, la mise en contact de la fraction de membrane avec un médicament dans des conditions permettant la liaison dudit médicament audit peptide et déterminant si le ledit médicament se lie de façon spécifique à la cellule transfectée, et ainsi identifiant un médicament qui se lie de façon spécifique audit peptide et qui peut être utilisé dans le traitement et/ou la prévention de l'infection au virus VIH.

6. Méthode pour déterminer si un agoniste ou antagoniste qui se lie à un peptide comprenant une séquence en acides aminés qui présente plus de 80 % d'homologie avec SEQ ID NO. 2 représentée sur la figure 1, ou une partie de celle-ci, peut être utilisée dans le traitement et/ou la prévention de l'infection au virus VIH, ladite méthode comprenant la mise en contact d'une cellule transfectée avec un vecteur exprimant la molécule d' acide nucléique codant ledit peptide avec l'agoniste ou l'antagoniste dans des conditions permettant la liaison de l'agoniste ou de l'antagoniste audit peptide et la détermination de savoir si ledit agoniste ou ledit antagoniste inhibe la liaison du virus VIH audit peptide.

7. Méthode pour déterminer si un agoniste ou antagoniste qui se lie à un peptide comprenant une séquence en acides aminés qui présente plus de 80 % d'homologie avec SEQ ID NO. 2 représentée sur la figure 1, ou une partie de celle-ci, peut être utilisée dans le traitement et/ou la prévention de l'infection au virus VIH, ladite méthode comprenant la préparation d'un extrait cellulaire à partir de cellules transfectées avec un vecteur exprimant la molécule d'acide nucléique codant ledit peptide, l'isolation d'une fraction de membrane à partir de l'extrait cellulaire, la mise en contact d'une fraction de membrane avec l'agoniste ou l'antagoniste dans des conditions permettant la liaison de l'agoniste ou de l'antagoniste audit peptide et la détermination de savoir si ledit agoniste ou ledit antagoniste inhibe la liaison du virus VIH audit peptide.

8. Méthode selon l'une des revendications 1 à 7, dans laquelle le virus VIH est choisi dans le groupe consistant en le virus d'immunodéficience humain 1 (VIH 1), le virus d'immunodéficience humain 2 (VIH 2) ou une portion desdits virus VIH.

9. Méthode selon l'une des revendications 1 à 8, dans laquelle la portion dudit virus VIH est la glycoprotéine GP 120/GP 160 ou une portion de celle-ci.

10. Méthode selon l'une des revendications 1 à 9, comprenant en outre l'étape de mesure de la capacité à être infecté de la cellule par une souche VIH dans laquelle ledit anti-ligand, médicament, agoniste ou antagoniste décroît la capacité à être infecté par ladite souche VIH.

11. Méthode selon la revendication 10, dans laquelle la cellule est une cellule lymphocyte.

12. Méthode selon l'une des revendications 10 ou 11, dans laquelle la souche du VIH est le virus d'immunodéficience humain 1 (VIH 1).

13. Méthode selon l'une des revendications 10 ou 11, dans laquelle la souche du VIH est le virus d'immunodéficience humain 2 (VIH 2).

14. Méthode selon l'une quelconque des revendications 10 à 13, dans laquelle la diminution du pouvoir infectieux de VIH est mesuré par le dosage d'une protéine VIH.

15. Méthode selon la revendication 14, dans laquelle ladite protéine du VIH est l'antigène P24 du VIH.

16. Méthode selon l'une quelconque des revendications 10 à 15, dans laquelle la séquence en acides aminés du peptide présente plus de 90 % d'homologie avec SEQ ID NO. 2 représentée sur la figure 1.

17. Méthode selon l'une quelconque des revendications 10 à 15, dans laquelle la séquence en acides aminés du peptide présente plus de 95 % d'homologie avec SEQ ID NO. 2 représentée sur la figure 1.

18. Méthode selon l'une quelconque des revendications 10 à 15, dans laquelle la séquence en acides aminés du peptide est la séquence en acides aminés SEQ ID NO. 2 représentée sur la figure 1 ou une portion de celle-ci comprenant au moins le segment N-terminal et la première boucle extracellulaire de la séquence en acides aminés SEQ ID NO. 2 représentée sur la figure 1.

19. Méthode selon la revendication 3, dans laquelle la modification dans l'activité du peptide est détectée par un test biologique basé sur la modification dans la production d'un second messager.

20. Méthode selon la revendication 19, dans laquelle le test biologique est basé sur la mesure des ions calcium ou sur la concentration des inositol phosphates (tels que IP_3).

21. Méthode selon l'une quelconque des revendications 1 à 20, dans laquelle la cellule est une cellule de mammifère d'origine non neuronale, de préférence choisie parmi le groupe consistant en cellules CHO-K1, HEK293, BHK21 et COS-7.

22. Méthode selon l'une quelconque des revendications 1 à 21, dans laquelle l'anti-ligand, le médicament, l'agoniste ou l'antagoniste est un anticorps.

23. Méthode selon la revendication 22, dans laquelle l'anticorps est un anticorps monoclonal.

24. Méthode selon la revendication 23, dans laquelle l'anticorps monoclonal est dirigé vers un épitope dudit peptide présent sur la surface de la cellule.

25. Anticorps qui inhibe ou réduit la liaison du virus d'immunodéficience humain 1 (VIH 1), ou du virus d'immunodéficience humain 2 (VIH 2) à un peptide ayant une séquence en acides aminés qui présente plus de 80 % d'homologie avec SEQ ID NO. 2 représentée sur la figure 1, identifié selon l'une quelconque des revendications 1 à 24.

26. Méthode selon la revendication 25, dans laquelle la séquence en acides aminés du peptide présente plus de 90 % d'homologie avec SEQ ID NO. 2 représentée sur la figure 1.

27. Méthode selon la revendication 25, dans laquelle la séquence en acides aminés du peptide présente plus de 95 % d'homologie avec SEQ ID NO. 2 représentée sur la figure 1.

28. Méthode selon la revendication 25, dans laquelle la séquence en acides aminés du peptide est la séquence en acides aminés SEQ ID NO. 2 représentée sur la figure 1.

29. Anticorps selon l'une quelconque des revendications 25 à 28, qui est un anticorps monoclonal.

30. Anticorps selon la revendications 29, qui est un anticorps monoclonal dirigé vers un épitope dudit peptide, présent sur la surface d'une cellule exprimant ledit peptide.

31. Anticorps selon l'une quelconque des revendications 25 à 30, qui décroît la capacité à être infecté d'une cellule par une souche de VIH.

32. Anticorps selon la revendication 31, dans lequel la cellule est une cellule lymphocyte.

33. Anticorps selon l'une quelconque des revendications 31 ou 32, dans lequel la souche du VIH est le virus d'immunodéficience humain 1 (VIH 1).

34. Anticorps selon l'une quelconque des revendications 31 ou 32, dans lequel la souche du VIH est le virus d'immunodéficience humain 2 (VIH 2).

35. Composition pharmaceutique comprenant un véhicule pharmaceutique acceptable et une quantité suffisante de l'anticorps selon l'une quelconque des revendications 25 à 34.

36. Utilisation de la composition pharmaceutique selon la revendication 35, pour la préparation d'un médicament pour le traitement et/ou la prévention d'une infection par le virus d'immunodéficience humain 1 (VIH 1) et/ou le virus d'immunodéficience humain 2 (VIH 2) (SIDA).

37. Utilisation d'un oligonucléotide antisens, comprenant une séquence qui s'hybride de façon spécifique à une molécule d'acide nucléique ayant plus de 80 % d'homologie avec SEQ ID NO. 2 représentée sur la figure 1, pour la préparation d'un médicament pour le traitement et/ou la prévention d'une infection par le virus d'immunodéficience humain 1 (VIH 1) et/ou le virus d'immunodéficience humain 2 (VIH 2) (SIDA).

38. Utilisation d'un oligonucléotide antisens, comprenant une séquence qui s'hybride de façon spécifique à une molécule d'acide nucléique ayant au moins la séquence d'acide nucléique SEQ ID NO. 2 représentée sur la figure 1 ou une partie de celle ci, pour la préparation d'un médicament pour le traitement et/ou la prévention d'une infection par le virus d'immunodéficience humain 1 (VIH 1) et/ou le virus d'immunodéficience humain 2 (VIH 2) (SIDA).

39. Utilisation selon lune des revendications 37 ou 38, dans laquelle la molécule d'acide nucléique code un peptide tel que défini dans l'une quelconque des revendications 1 à 7 et 16 à 18.

40. Utilisation selon les revendications 37 à 38, dans laquelle la molécule d'acide nucléique est une molécule d'ADNc ou une molécule d'ADN génomique.

41. Utilisation selon les revendications 37 à 38, dans laquelle ledit oligonucléotide antisens comprend des analogues chimiques de nucléotides.

SEQ ID NO.1

FIG.1 a

GAATTCCCCCAACAGAGCCAAGCTCTCCATCTAGTGGACAGGGAAGCTAGCAGCAAACC 59
19

TTCCCTTCACTACAAAACTTCATTGCTTGGCCAAAAAGAGAGTTAATTCAATGTAGACAT 119
39

CTATGTAGGCAATTAAAAACCTATTGATGTATAAAACAGTTTGCATTCATGGAGGGCAAC 179
59

TAAATACATTCTAGGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAG 239
79

ATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGAGCCCTGC 299
M D Y Q V S S P I Y D I N Y Y T S E P C 99

CAAAAAATCAATGTGAAGCAAATCGCAGCCCGCCTCCTGCCTCCGCTCTACTCACTGGTG 359
Q K I N V K Q I A A R L L P P L Y S L V 119

TTCATCTTTGGTTTTGTGGGCAACATGCTGGTCATCCTCATCCTGATAAACTGCAAAAGG 419
F I F G F V G N M L V I L I L I N C K R 139

CTGAAGAGCATGACTGACATCTACCTGCTCAACCTGGCCATCTCTGACCTGTTTTTCCTT 479
L K S M T D I Y L L N L A I S D L F F L 159

CTTACTGTCCCCTTCTGGGCTCACTATGCTGCCGCCCAGTGGGACTTTGGAAATACAATG 539
L T V P F W A H Y A A A Q W D F G N T M 179

TGTCAACTCTTGACAGGGCTCTATTTTATAGGCTTCTTCTCTGGAATCTTCTTCATCATC 599
C Q L L T G L Y F I G F F S G I F F I I 199

CTCCTGACAATCGATAGGTACCTGGCTGTCGTCCATGCTGTGTTTGCTTTAAAAGCCAGG 659
L L T I D R Y L A V V H A V F A L K A R 219

ACGGTCACCTTTGGGGTGGTGACAAGTGTGATCACTTGGGTGGTGGCTGTGTTTGCGTCT 719
T V T F G V V T S V I T W V V A V F A S 239

CTCCCAGGAATCATCTTTACCAGATCTCAAAAAGAAGGTCTTCATTACACCTGCAGCTCT 779
L P G I I F T R S Q K E G L H Y T C S S 259

CATTTTCCATACA
H F P Y

```
GAATTCCCCCAACAGAGCCAAGCTCTCCATCTAGTGGACAGGGAAGCTAGCAGCAAACC   59
                                                              19

TTCCCTTCACTACAAAACTTCATTGCTTGGCCAAAAAGAGAGTTAATTCAATGTAGACAT 119
                                                              39

CTATGTAGGCAATTAAAAACCTATTGATGTATAAAACAGTTTGCATTCATGGAGGGCAAC 179
                                                              59

TAAATACATTCTAGGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAG 239
                                                              79

ATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGAGCCCTGC 299
 M  D  Y  Q  V  S  S  P  I  Y  D  I  N  Y  Y  T  S  E  P  C   99

CAAAAAATCAATGTGAAGCAAATCGCAGCCCGCCTCCTGCCTCCGCTCTACTCACTGGTG 359
 Q  K  I  N  V  K  Q  I  A  A  R  L  L  P  P  L  Y  S  L  V  119

TTCATCTTTGGTTTTGTGGGCAACATGCTGGTCATCCTCATCCTGATAAACTGCAAAAGG 419
 F  I  F  G  F  V  G  N  M  L  V  I  L  I  L  I  N  C  K  R  139

CTGAAGAGCATGACTGACATCTACCTGCTCAACCTGGCCATCTCTGACCTGTTTTTCCTT 479
 L  K  S  M  T  D  I  Y  L  L  N  L  A  I  S  D  L  F  F  L  159

CTTACTGTCCCCTTCTGGGCTCACTATGCTGCCGCCCAGTGGGACTTTGGAAATACAATG 539
 L  T  V  P  F  W  A  H  Y  A  A  A  Q  W  D  F  G  N  T  M  179

TGTCAACTCTTGACAGGGCTCTATTTTATAGGCTTCTTCTCTGGAATCTTCTTCATCATC 599
 C  Q  L  L  T  G  L  Y  F  I  G  F  F  S  G  I  F  F  I  I  199

CTCCTGACAATCGATAGGTACCTGGCTGTCGTCCATGCTGTGTTTGCTTTAAAAGCCAGG 659
 L  L  T  I  D  R  Y  L  A  V  V  H  A  V  F  A  L  K  A  R  219

ACGGTCACCTTTGGGGTGGTGACAAGTGTGATCACTTGGGTGGTGGCTGTGTTTGCGTCT 719
 T  V  T  F  G  V  V  T  S  V  I  T  W  V  V  A  V  F  A  S  239

CTCCCAGGAATCATCTTTACCAGATCTCAAAAAGAAGGTCTTCATTACACCTGCAGCTCT 779
 L  P  G  I  I  F  T  R  S  Q  K  E  G  L  H  Y  T  C  S  S  259

CATTTTCCATACAGTCAGTATCAATTCTGGAAGAATTTCCAGACATTAAAGATAGTCATC 839
 H  F  P  Y  S  Q  Y  Q  F  W  K  N  F  Q  T  L  K  I  V  I  279
```

SEQ ID NO.2 FIG.1b

```
TTGGGGCTGGTCCTGCCGCTGCTTGTCATGGTCATCTGCTACTCGGGAATCCTAAAAACT   899
 L  G  L  V  L  P  L  L  V  M  V  I  C  Y  S  G  I  L  K  T    299

CTGCTTCGGTGTCGAAATGAGAAGAAGAGGCACAGGGCTGTGAGGCTTATCTTCACCATC   959
 L  L  R  C  R  N  E  K  K  R  H  R  A  V  R  L  I  F  T  I    319

ATGATTGTTTATTTTCTCTTCTGGGCTCCCTACAACATTGTCCTTCTCCTGAACACCTTC  1019
 M  I  V  Y  F  L  F  W  A  P  Y  N  I  V  L  L  L  N  T  F    339

CAGGAATTCTTTGGCCTGAATAATTGCAGTAGCTCTAACAGGTTGGACCAAGCTATGCAG  1079
 Q  E  F  F  G  L  N  N  C  S  S  S  N  R  L  D  Q  A  M  Q    359

GTGACAGAGACTCTTGGGATGACGCACTGCTGCATCAACCCCATCATCTATGCCTTTGTC  1139
 V  T  E  T  L  G  M  T  H  C  C  I  N  P  I  I  Y  A  F  V    379

GGGGAGAAGTTCAGAAACTACCTCTTAGTCTTCTTCCAAAAGCACATTGCCAAACGCTTC  1199
 G  E  K  F  R  N  Y  L  L  V  F  F  Q  K  H  I  A  K  R  F    399

TGCAAATGCTGTTCTATTTTCCAGCAAGAGGCTCCCGAGCGAGCAAGCTCAGTTTACACC  1259
 C  K  C  C  S  I  F  Q  Q  E  A  P  E  R  A  S  S  V  Y  T    419

CGATCCACTGGGGAGCAGGAAATATCTGTGGGCTTGTGACACGGACTCAAGTGGGCTGGT  1319
 R  S  T  G  E  Q  E  I  S  V  G  L  *                        439

GACCCAGTCAGAGTTGTGCACATGGCTTAGTTTTCATACACAGCCTGGGCTGGGGGTNGG  1379
                                                               459

TTGGNNGAGGTCTTTTTTAAAAGGAAGTTACTGTTATAGAGGGTCTAAGATTCATCCATT  1439
                                                               479

TATTTGGCATCTGTTTAAAGTAGATTAGATCCGAATTC
```

SEQ ID NO.2 (SUITE)

FIG.1c

```
GAATTCCCCCAACAGAGCCAAGCTCTCCATCTAGTGGACAGGGAAGCTAGCAGCAAACC   59
                                                               19

TTCCCTTCACTACAAAACTTCATTGCTTGGCCAAAAAGAGAGTTAATTCAATGTAGACAT 119
                                                               39

CTATGTAGGCAATTAAAAACCTATTGATGTATAAAACAGTTTGCATTCATGGAGGGCAAC 179
                                                               59

TAAATACATTCTAGGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAG 239
                                                               79

ATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGAGCCCTGC 299
 M  D  Y  Q  V  S  S  P  I  Y  D  I  N  Y  Y  T  S  E  P  C    99

CAAAAAATCAATGTGAAGCAAATCGCAGCCCGCCTCCTGCCTCCGCTCTACTCACTGGTG 359
 Q  K  I  N  V  K  Q  I  A  A  R  L  L  P  P  L  Y  S  L  V   119

TTCATCTTTGGTTTTGTGGGCAACATGCTGGTCATCCTCATCCTGATAAACTGCAAAAGG 419
 F  I  F  G  F  V  G  N  M  L  V  I  L  I  L  I  N  C  K  R   139

CTGAAGAGCATGACTGACATCTACCTGCTCAACCTGGCCATCTCTGACCTGTTTTTCCTT 479
 L  K  S  M  T  D  I  Y  L  L  N  L  A  I  S  D  L  F  F  L   159

CTTACTGTCCCCTTCTGGGCTCACTATGCTGCCGCCCAGTGGGACTTTGGAAATACAATG 539
 L  T  V  P  F  W  A  H  Y  A  A  A  Q  W  D  F  G  N  T  M   179

TGTCAACTCTTGACAGGGCTCTATTTTATAGGCTTCTTCTCTGGAATCTTCTTCATCATC 599
 C  Q  L  L  T  G  L  Y  F  I  G  F  F  S  G  I  F  F  I  I   199

CTCCTGACAATCGATAGGTACCTGGCTGTCGTCCATGCTGTGTTTGCTTTAAAAGCCAGG 659
 L  L  T  I  D  R  Y  L  A  V  V  H  A  V  F  A  L  K  A  R   219

ACGGTCACCTTTGGGGTGGTGACAAGTGTGATCACTTGGGTGGTGGCTGTGTTTGCGTCT 719
 T  V  T  F  G  V  V  T  S  V  I  T  W  V  V  A  V  F  A  S . 239

CTCCCAGGAATCATCTTTACCAGATCTCAAAAAGAAGGTCTTCATTACACCTGCAGCTCT 779
 L  P  G  I  I  F  T  R  S  Q  K  E  G  L  H  Y  T  C  S  S   259

CATTTTCCATACATTAAAGATAGTCATCTTGGGGCTGGTCCTGCCGCTGCTTGTCATGGT 839
 H  F  P  Y  I  K  D  S  H  L  G  A  G  P  A  A  A  C  H  G   279
```

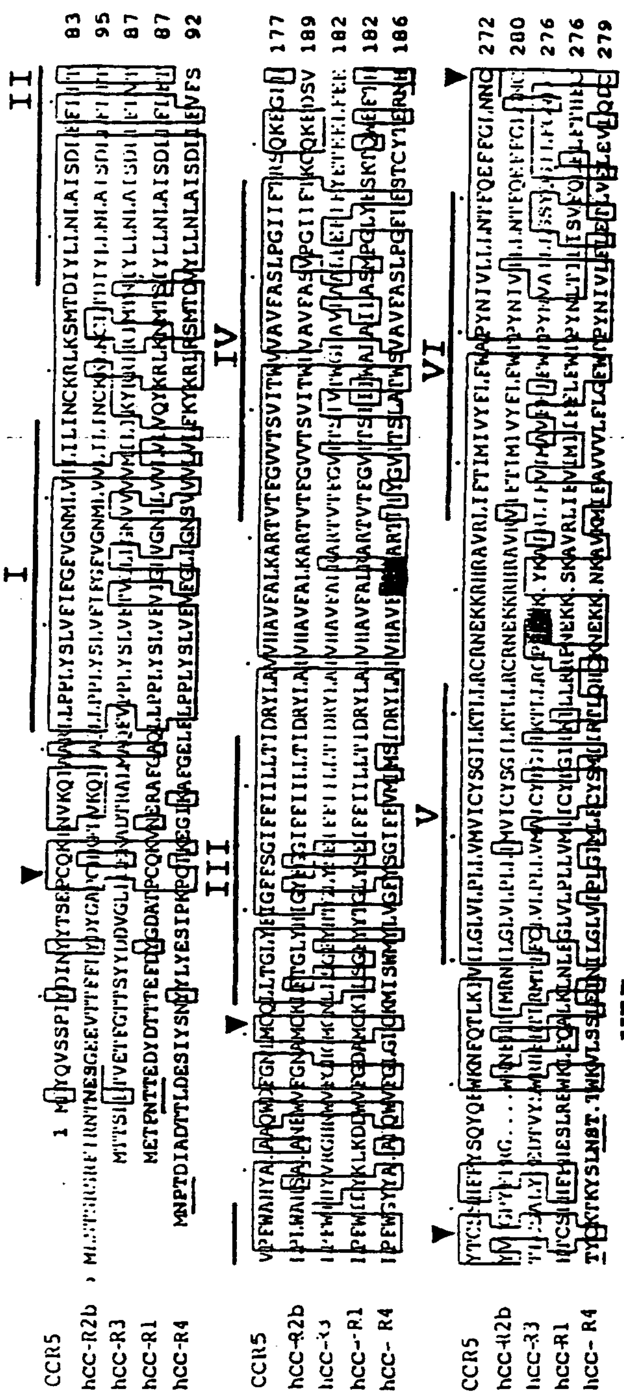
SEQ ID NO.3 FIG.1d

```
CATCTGCTACTCGGGAATCCTAAAAACTCTGCTTCGGTGTCGAAATGAGAAGAAGAGGCA    899
 H  L  L  L  G  N  P  K  N  S  A  S  V  S  K  *                 299

CAGGGCTGTGAGGCTTATCTTCACCATCATGATTGTTTATTTTCTCTTCTGGGCTCCCTA    959
                                                                319

CAACATTGTCCTTCTCCTGAACACCTTCCAGGAATTCTTTGGCCTGAATAATTGCAGTAG   1019
                                                                339

CTCTAACAGGTTGGACCAAGCTATGCAGGTGACAGAGACTCTTGGGATGACGCACTGCTG   1079
                                                                359

CATCAACCCCATCATCTATGCCTTTGTCGGGGAGAAGTTCAGAAACTACCTCTTAGTCTT   1139
                                                                379

CTTCCAAAAGCACATTGCCAAACGCTTCTGCAAATGCTGTTCTATTTTCCAGCAAGAGGC   1199
                                                                399

TCCCGAGCGAGCAAGCTCAGTTTACACCCGATCCACTGGGGAGCAGGAAATATCTGTGGG   1259
                                                                419

CTTGTGACACGGACTCAAGTGGGCTGGTGACCCAGTCAGAGTTGTGCACATGGCTTAGTT   1319
                                                                439

TTCATACACAGCCTGGGCTGGGGGTNGGTTGGNNGAGGTCTTTTTTAAAAGGAAGTTACT   1379
                                                                459

GTTATAGAGGGTCTAAGATTCATCCATTTATTTGGCATCTGTTTAAAGTAGATTAGATCC   1439
                                                                479

GAATTC
```

SEQ ID NO.3 (SUITE)

FIG.1e

FIG. 2



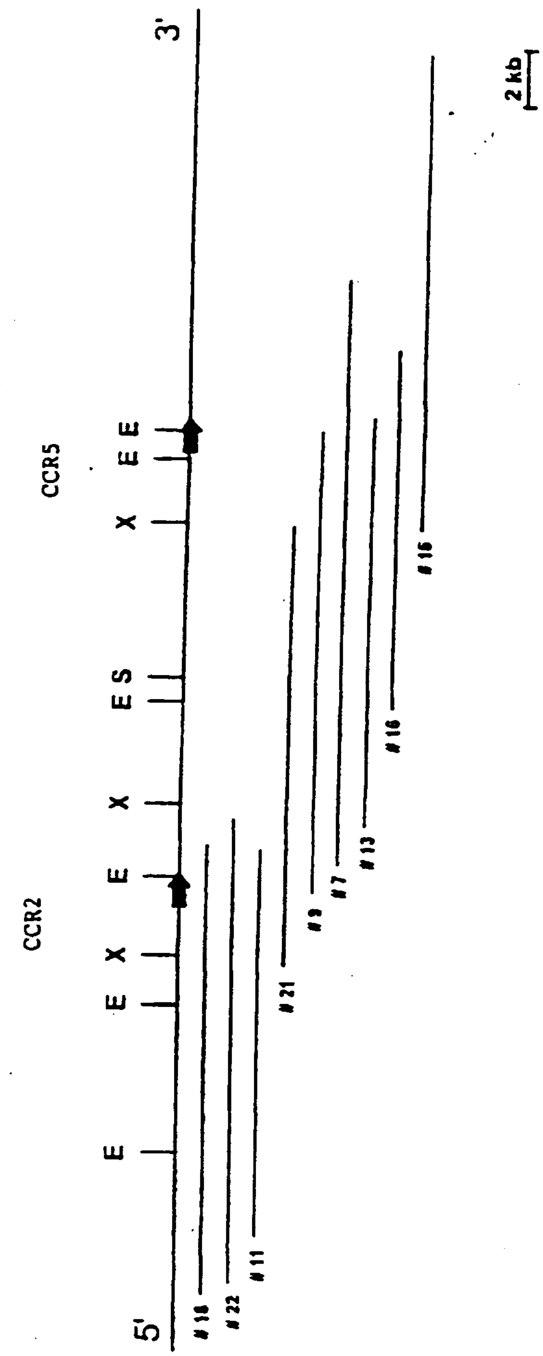


FIG.3

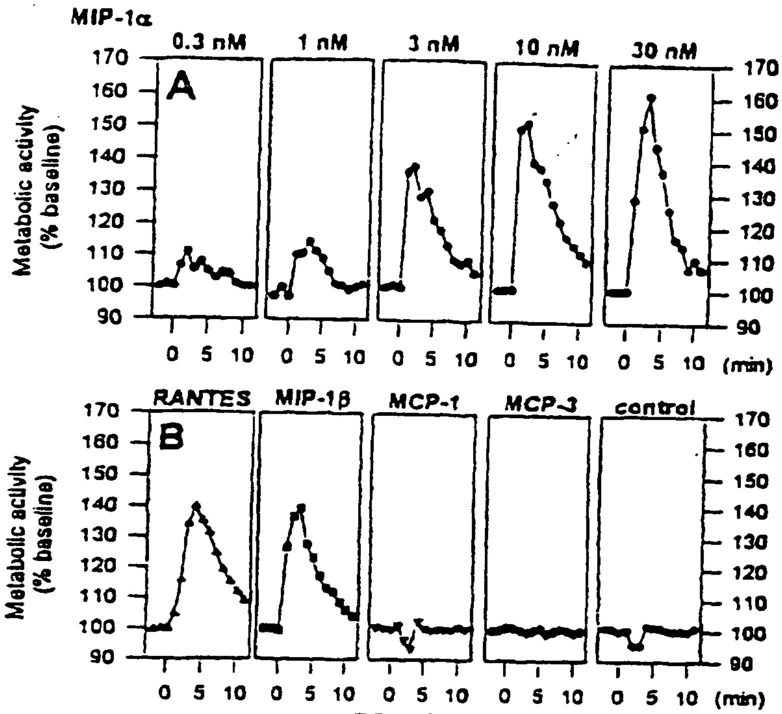

MIP-1α
0.3 nM
1 nM
3 nM
10 nM
30 nM
A
Metabolic activity (% baseline)
(min)
RANTES
MIP-1β
MCP-1
MCP-3
control
B
Metabolic activity (% baseline)
(min)

FIG.4a

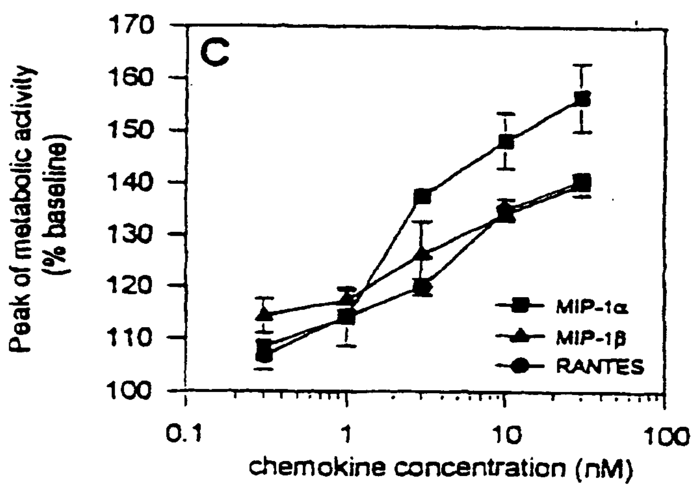

C
Peak of metabolic activity (% baseline)
MIP-1α
MIP-1β
RANTES
chemokine concentration (nM)

FIG.4b

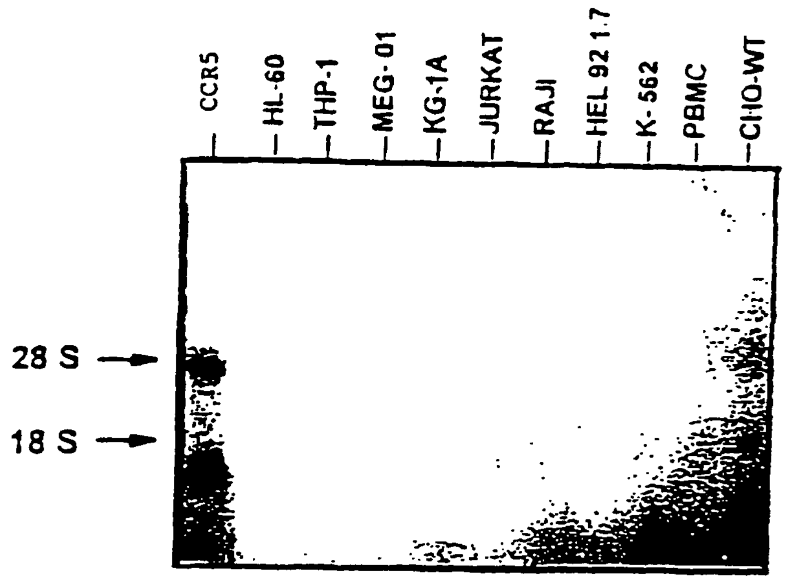
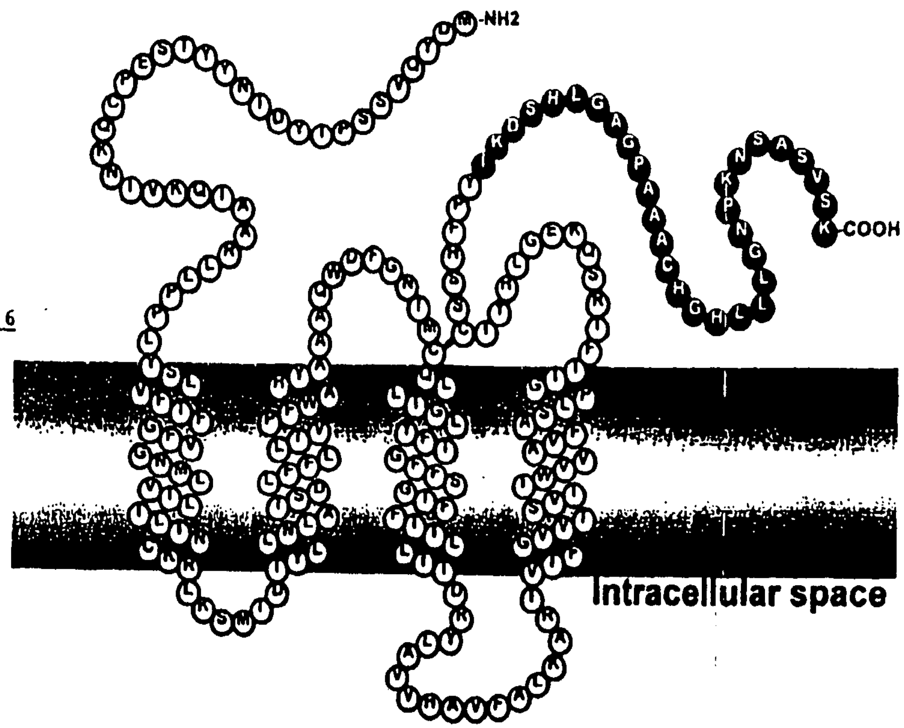

CCR5
HL-60
THP-1
MEG-01
KG-1A
JURKAT
RAJI
HEL 92 1.7
K-562
PBMC
CHO-WT
28 S
18 S

FIG.5

FIG. 6

a



b

```
CCR5   F  P  Y  S  Q  Y  Q  F  W  K  N  F  Q  T  L  K  I  V  I  L  G  L  V  L  P
       TTTCCATACAgtcagtatcaattctggaagaatttccagacaTTAAAGATAGTCATCTTGGGGCTGGTCCTGCCG
Δccr5  F  P  Y                 deletion              I  K  D  S  H  L  G  A  G  P  A

CCR5   L  L  V  M  V  I  C  Y  S  G  I  L  K  T  L  L  R  C  R  N  E  K  K  R
       CTGCTTGTCATGGTCATCTGCTACTCGGGAATCCTAAAAACTCTGCTTCGGTGTCGAAATGAGAAGAAGAGG
Δccr5  A  A  C  H  G  H  L  L  L  G  N  P  K  N  S  A  S  V  S  K  *
```

A.

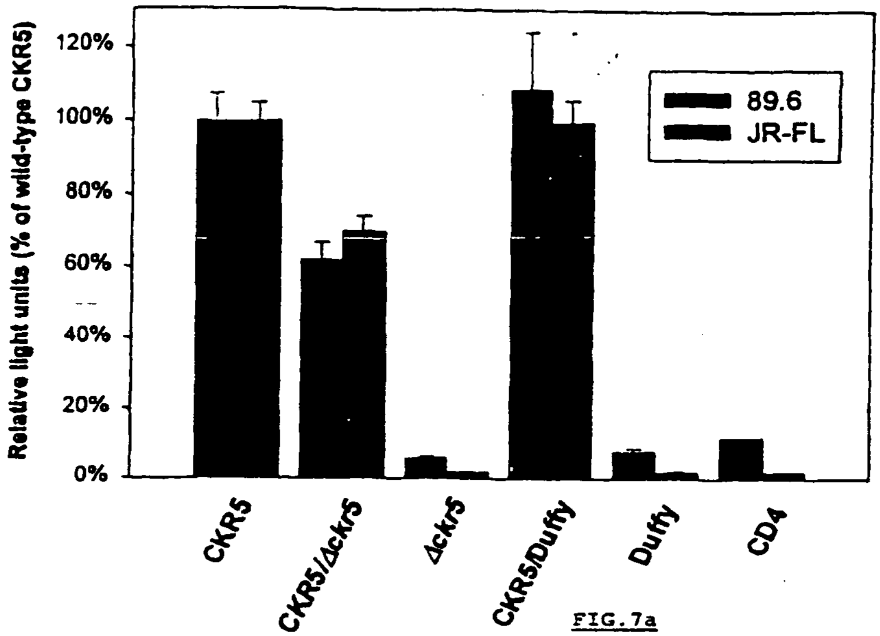


FIG. 7a

B.

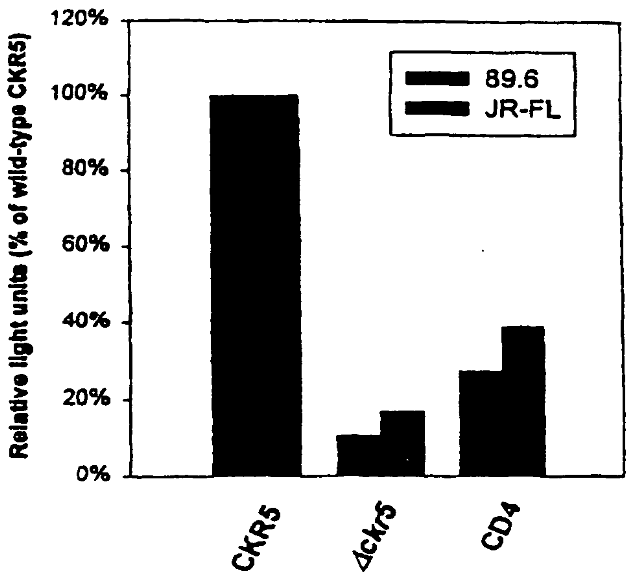


FIG. 7b

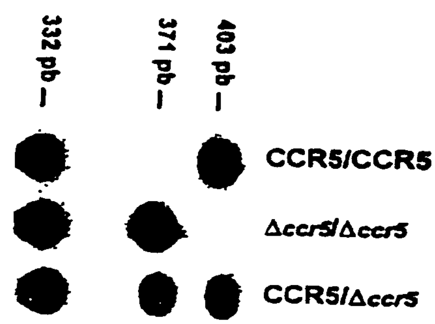


FIG.8

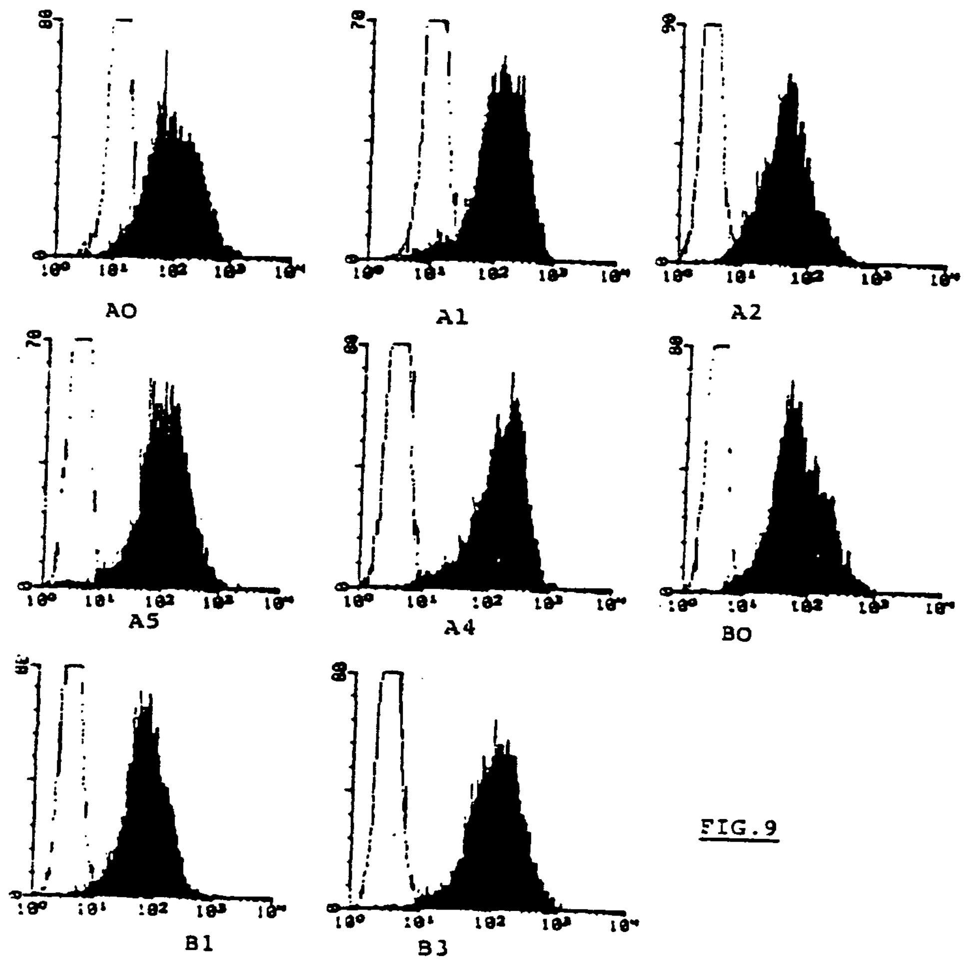


FIG. 9

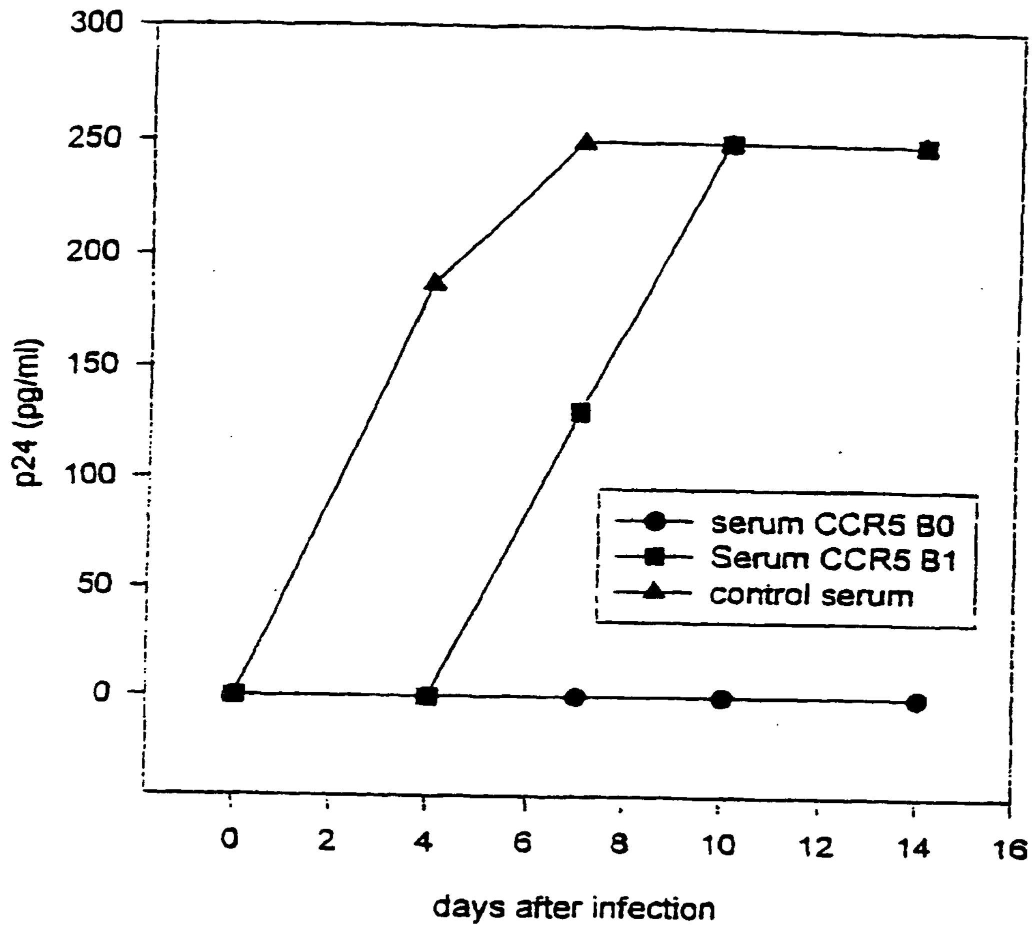

300
250
200
150
100
50
0
p24 (pg/ml)
0
2
4
6
8
10
12
14
16
days after infection
serum CCR5 B0
Serum CCR5 B1
control serum

FIG.10